

ANNEX B

Active substance: Bensulfuron methyl

B.7 Residue data

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table of contents

B.7	Residue data	3
B.7.1	Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1)	3
B.7.1.1	Rice metabolism study	3
B.7.1.2	Overall conclusions on nature of the plant residues	8
B.7.2	Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1)	8
B.7.2.1	Goat metabolism study	9
B.7.2.2	Laying hen metabolism study	17
B.7.2.3	Overall conclusions on metabolism in animals	23
B.7.3	Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)	25
B.7.4	Use pattern	25
B.7.5	Identification of critical GAPs	27
B.7.6	Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)	27
B.7.6.1	Stability of residues during storage of samples: rice grain and rice straw freezer storage stability studies	27
B.7.6.2	Supervised residue trials	31
B.7.6.3	Overall conclusions on residues resulting from supervised trials	43
B.7.7	Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)	45
B.7.8	Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)	45
B.7.9	Residues in succeeding or rotational crops (Annex IIA 6.6; Annex III 8.5)	46
B.7.10	Proposed pre-harvest intervals for envisaged uses, or withholding periods or storage periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7)	50
B.7.10.1	Proposed pre-harvest intervals (PHIs)	50
B.7.11	Community MRLs and MRLs in EU Member States (Annex IIIA 12.2)	50
B.7.12	Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)	51
B.7.13	Proposed EU import tolerances and justification for acceptability of those residues (Annex IIA 6.7 and IIIA 8.6)	51
B.7.14	Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs	51
B.7.15	Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)	52
B.7.15.1	Chronic exposure through diet	52
B.7.15.2	Short-term dietary risk assessment	53
B.7.15.3	Overall conclusions on dietary exposure	54
B.7.16	Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)	54
B.7.16.1	Plant metabolism	54
B.7.16.2	Livestock metabolism	54
B.7.16.3	Residues resulting from supervised trials	55
B.7.16.4	Feeding studies in livestock	55
B.7.16.5	Effects of industrial processing and/or household preparation on the nature and magnitude of residues	56
B.7.16.6	Residue data in succeeding or rotational crops	56
B.7.16.7	Proposed maximum residue levels (MRLs)	56
B.7.16.8	Proposed pre-harvest intervals for envisaged uses	56
B.7.16.9	Dietary exposure	56
B.7.17	References relied on	58

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7 Residue data

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1)

The comparative metabolism of bensulfuron methyl (DPX-F5384) study in plants was conducted in rice. This crop represents the current targeted uses of this product. Bensulfuron methyl is a selective rice herbicide for use at a maximum seasonal rates of 60 g a.s./ha. Rice, grown in paddies, was treated with [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl at a rate of 90 g a.s./ha (normal use rate is 60 g a.s./ha), then harvested at various time intervals following treatment. The distribution of total ¹⁴C residues in/on the raw agricultural commodities (e.g. straw and grain) was determined. Characterisation of significant ¹⁴C-residues (accounting for >10% of the total residues) and the elucidation of the metabolic pathway of ¹⁴C bensulfuron methyl in rice plants were carried out.

In order to support the intended uses of bensulfuron in rice, one metabolism study was carried out in rice grown in paddies. The total radioactive residues in rice grown in paddies was negligible after treatment with bensulfuron methyl at 90 g a.s./ha (approximately 1.5 times the maximum total annual GAP use rate). The total radioactive residues in rice straw and grain were very low (0.003 to 0.009 mg/kg and 0.003 mg/kg, respectively). The primary metabolic pathway was *O*-desmethylation yielding *O*-desmethyl bensulfuron methyl (IN-F7880). Another pathway involved the cleavage of the sulfonylurea bridge resulting in sulphonamide (IN-N5297) and pyrimidine amine (IN-J0290). The sulphonamide undergoes cyclisation to yield homosaccharin (IN-B6895).

B.7.1.1 Rice metabolism study

- 1) *Metabolism of [phenyl(U)-¹⁴C]DPX-F5384 and [pyrimidine-2-¹⁴C]DPX-F5384 in field-grown Japonica rice.*
Hunt, O.R., Berus, J.S., 1986. Dupont unpublished report No. AMR 473-86. Dates of experimental work:
Initiation date: June 22, 1984.

Cross reference IIA, 6.1./01

Guidelines:

USEPA Pesticide Assessment Guidelines Subdivision N, Document No. 83-153976

Deviations: None

GLP:

No GLP was required at the time of the study.

The study is acceptable.

Materials and Methods:

Test Substance: ¹⁴C-DPX-F5384 technical

Batch: [Phenyl(U)-¹⁴C] Batch number not specified, [Pyrimidine-2-¹⁴C] Batch number not specified

Purity: >99% radiochemical purity

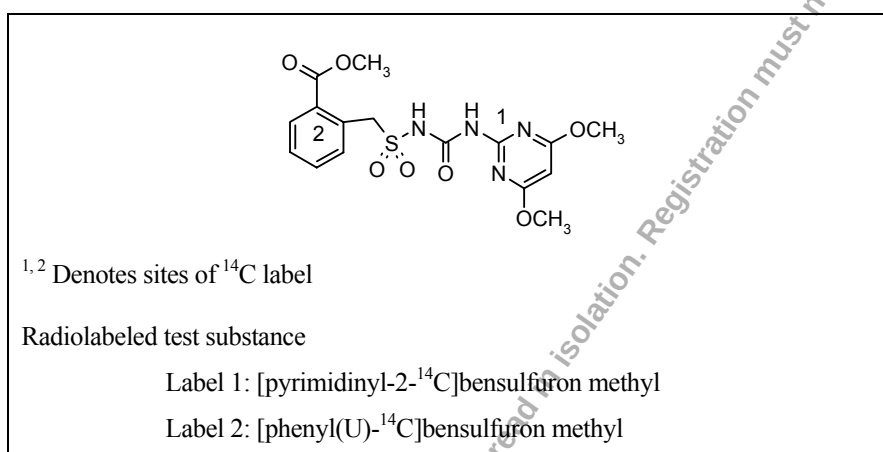
The metabolism of bensulfuron methyl, using two radiolabeled test substances (Figure B.7. 1), [phenyl(U)-¹⁴C]bensulfuron (radiochemical purity >99%; 29.8 µCi/mg) and [pyrimidine-2-¹⁴C]bensulfuron methyl

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

(radiochemical purity >99%; 16.6 $\mu\text{Ci}/\text{mg}$) at 90 g a.s./ha, in Japonica rice plants grown in field paddies was investigated. A granular formulation of each radiolabeled test substance containing formulating inert ingredients was prepared for the treatment of the rice paddies. Two paddies were treated separately with [phenyl(U)- ^{14}C]bensulfuron methyl and two more paddies with [pyrimidine-2- ^{14}C]bensulfuron methyl. The granular formulated products were sprinkled evenly over the water surface of each paddy.

Figure B.7. 1: Structure of radiolabeled bensulfuron methyl



Samples of rice plants, paddy soil and paddy water were taken for analysis throughout the duration of the study. The sample schedules were: Pre-treatment, 30, 60, and 90 days after the treatment, and maturity (99 days). Control water was taken from the reservoir, and control soil was taken from the area immediately surrounding the outer rectangles at the same intervals.

Radioactivity in water samples was determined by liquid scintillation counting (LSC). The soil samples were air-dried, ground into a fine powder, homogenised, and combusted to determine the total radioactive residues. The foliage and grain were separated, freeze-dried, ground and homogenised. Triplicate aliquots were analysed for total radioactivity by combustion analysis followed by LSC. The total radioactive residues in the foliage samples collected at various intervals (Day 30 to Day 99) and the grain (Day 99) are summarised in Table B.7. 1.

The homogenised foliage samples (100-200 g) were washed sequentially with chloroform and acetonitrile (Table B.7. 2). The plant tissue was then extracted with acetone:water (1:1, v/v) for 2-3 minutes using a Waring Blendor®. The plant matrix was separated from the extract by filtration and the extraction process was repeated three more times. The combined extracts were concentrated under vacuum and the slurry was centrifuged and the supernatant extract decanted from remaining solids. The amount of radioactivity in each foliage extract, including the chloroform and acetonitrile wash solutions from various sampling intervals were determined by LSC. The unextracted radioactivity in the plant samples was determined by combustion analysis of the extracted plant samples, followed by LSC analysis.

The foliage acetone/water extract was adjusted to pH 10 with 28% ammonium hydroxide solution and extracted with n-hexane (4 x 500 mL) to remove green pigments and non-polar natural products. Then the extract was partitioned with methylene chloride, which removed more non-polar natural products. The aqueous solution was then neutralised to pH 7 using phosphoric acid and partitioned with methylene chloride (3 x 500 mL). The aqueous extract was then acidified to pH 3 with phosphoric acid and partitioned with methylene chloride (3 x 500 mL). The methylene

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

chloride extracts at pH 7 and 3 were pooled separately. The amount of radioactivity in the pooled methylene chloride extracts was determined by LSC. The chloroform and n-hexane extracts were discarded, as they contained negligible ^{14}C residues (Table B.7. 2). The composite acetonitrile wash, the two composite methylene chloride fractions, and the aqueous fraction were each concentrated to near dryness under reduced pressure using a rotary evaporator (30–40°C). The residual acetonitrile and methylene chloride fractions were reconstituted with 10 mL each of the corresponding fresh solvents and filtered. The volume of the filtrate was reduced to 2 mL by evaporation under a stream of nitrogen. The residues from the aqueous fraction were reconstituted in methanol. Natural products were precipitated by the addition of acetone to the methanol solution. Precipitated solids were removed by centrifugation. The acetone was removed by rotary evaporation (approximately 40°C). The methanol solution was filtered and concentrated to 2 mL under a stream of nitrogen. Extracts containing significant ^{14}C residues were analysed by thin layer chromatography (TLC).

Aliquots (50 to 100 μL) of processed extracts from foliage and grain were applied to the TLC plates. Two TLC methods were used. One method used 0.25 mm silica gel TLC plates and methylene chloride:methanol:ammonium hydroxide (150:50:3, v/v/v). The plates were developed for a distance of 15 cm. The second method used 0.25 mm two phase plates (Whatman CS5[®]) and methylene chloride:acetonitrile:water:acetic acid (150:27:0.3:2.7, v/v/v/v). These plates were also developed for 15 cm (1 cm of reverse phase and 14 cm of silica gel GF).

Radiolabeled metabolites were identified by co-chromatography with the reference standards. Amounts of metabolites in each sample were determined by integrating the areas of the radioactive zones.

Findings:

Distribution of residues: Dislodgeable surface ^{14}C residues were not expected from the rice foliage, as the granular formulation of the test substance was applied to the water surface of the rice paddies. The TRR in all the foliage samples collected at various intervals were in a range of 0.0003 mg/kg to 0.009 mg/kg. The total radioactive residues (TRR) in the grain from [phenyl(U)- ^{14}C]bensulfuron methyl and [pyrimidine-2- ^{14}C]bensulfuron methyl were 0.003 mg/kg.

Characterisation of residues: The TRR in the rice grain was low (0.003 mg/kg) in both radiolabeled samples. Less than 50% of the TRR was extracted into acetone/water. No discrete metabolites were detected in the extracts when analysed by TLC. Extracted and unextracted ^{14}C residues from the foliage samples are summarised in Table B.7. 2. The ^{14}C residues in the aqueous acetone extracts were subjected to solvent-solvent partitioning and the results are summarised in Table B.7. 3.

Metabolites were tentatively characterised using foliage samples. No intact bensulfuron methyl was detected by thin layer chromatography (TLC). *O*-desmethyl bensulfuron methyl (IN-F7880), homosaccharin (IN-B6895), sulphonamide (IN-N5297), and pyrimidine amine (IN-J0290) were characterised by co-chromatography with reference standards. The levels of individual metabolites were insignificant and accurate quantitation was not possible. Approximate concentrations of individual metabolites are provided in Table B.7. 4.

Metabolic Pathway:

Primary metabolic pathway of bensulfuron methyl in Japonica rice plants (see Figure B.7. 2:) is *O*-demethylation of the methoxypyrimidine moiety to yield *O*-desmethyl bensulfuron methyl (IN-F7880). Bensulfuron methyl also undergoes cleavage of sulfonylurea linkage to yield sulphonamide (IN-N5297) and pyrimidine amine (IN-J0290). The sulphonamide cyclises to yield homosaccharin (IN-B6895).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Conclusion:

Japonica rice grown in paddies treated with either [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl at 90 g a.s./ha absorbed only negligible quantities of total radioactive residues. The TRR in rice grain was 0.003 mg/kg, whereas the foliage samples at various intervals contained 0.003-0.009 mg/kg. Despite the low level of total radioactive residues, the metabolites were tentatively characterised. Rice straw at maturity did not contain bensulfuron methyl. *O*-Demethylation yielding IN-F7880 was the major metabolic pathway. The other pathway included cleavage of the sulfonylurea bridge, resulting in sulphonamide (IN-N5297) and pyrimidine amine (IN-J0290). The sulphonamide undergoes cyclisation to yield homosaccharin (IN-B6895).

Table B.7. 1: Distribution of ¹⁴C bensulfuron methyl equivalent residues in rice foliage and grain at various time intervals after the initial application

	[Phenyl(U)- ¹⁴ C]bensulfuron methyl	[Pyrimidine-2- ¹⁴ C]bensulfuron methyl
Foliage	mg/kg^a	mg/kg^a
Day 0 ^b	NA	NA
Day 30	0.005	0.003
Day 60	0.006	0.009
Day 90	0.005	0.009
Day 99	0.004	0.007
Grain	mg/kg	mg/kg
Day 99	0.003	0.003

^a mg/kg values expressed on a tissue wet weight basis

^b Day 0 water contained 0.034 mg/kg, water from all other sampling intervals contained <0.002 mg/kg

^c Not applicable; no sample

Table B.7. 2: Extraction of radioactivity from rice foliage treated with [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl at 90 g a.s./ha

Wash/extract	[Phenyl(U)- ¹⁴ C]bensulfuron methyl			
	(% Total radioactive residues)			
	Day 30	Day 60	Day 90	Day 99
Chloroform	2.6	ND	7.7	ND
Acetonitrile	8.6	2.6	30.6	1.5
Acetone/water	77.7	73.2	40.1	57.2
Unextracted	11.1	24.2	21.6	41.3
Total	100.0	100.0	100.0	100
Wash/extract	[Pyrimidine-2- ¹⁴ C]bensulfuron methyl			
	(% Total radioactive residues)			
	Day 30	Day 60	Day 90	Day 99
Chloroform	2.6	ND	ND	ND
Acetonitrile	11.6	1.7	1.8	3.4
Acetone/water	76.6	54.0	48.7	44.2
Unextracted	9.2	44.3	49.5	52.4
Total	100.0	100.0	100.0	100.0

ND = not detected (1% TRR)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 3: Extraction of radioactivity from the acetone/water extract from rice foliage treated with [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl at 90 g a.s./ha

Wash/extract	[Phenyl(U)- ¹⁴ C]bensulfuron methyl			
	(%Total radioactive residues)			
	Day 30	Day 60	Day 90	Day 99
Acetone/water	77.7	73.2	40.1	57.2
n-Hexane	ND	6.1	ND	ND
CH ₂ Cl ₂ at pH 7	4.6	2.3	3.1	4.4
CH ₂ Cl ₂ at pH 3	31.8	20.7	10.2	11.0
Aqueous	41.3	44.0	26.8	41.8
Mass Balance % TRR (% acetone/water) ^a	77.7 (100)	73.1 (100)	40.1 (100)	57.2 (100)
Extract	[Pyrimidine-2- ¹⁴ C]bensulfuron methyl			
	(% Total radioactive residues)			
	Day 30	Day 60	Day 90	Day 99
Acetone/water	76.6	54.0	48.7	44.2
n-Hexane	ND	6.5	ND	ND
CH ₂ Cl ₂ at pH 7	9.1	1.5	12.5	13.0
CH ₂ Cl ₂ at pH 3	20.2	6.9	4.7	4.4
Aqueous	47.4	39.1	31.6	26.8
Mass Balance % TRR (% acetone/water) ^a	76.6 (100)	54.0 (100)	48.7 (100)	44.2 (100)

ND = not detected (1% TRR)

^a The results were normalised to 100% of the ¹⁴C residues in the acetone/water extract.

Table B.7. 4: Radioactive residues in the rice foliage after treatment with [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl

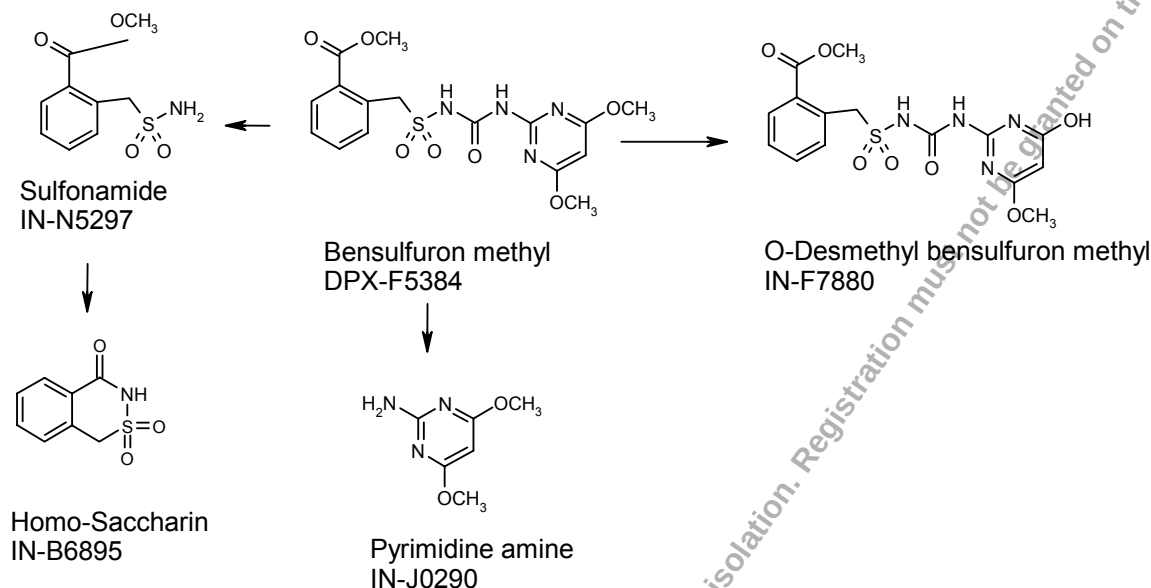
¹⁴ C residues in the foliage tissue	Concentration of [Phenyl(U)- ¹⁴ C]bensulfuron methyl equivalent ¹⁴ C residues, mg/kg				Concentration of [Pyrimidine-2- ¹⁴ C]bensulfuron methyl equivalent ¹⁴ C residues, mg/kg			
	Day 30	Day 60	Day 90	Day 99	Day 30	Day 60	Day 90	Day 99
Bensulfuron methyl	ND	ND	ND	ND	ND	ND	ND	ND
IN-F7880	0.0005	0.0005	0.0005	ND	0.0006	ND	ND	ND
IN-N5297	0.0005	ND	0.0012	ND	ND	ND	ND	ND
IN-B6895	0.0005	0.0005	0.0008	0.0006	NA	NA	NA	NA
IN-J0290	NA	NA	NA	NA	0.0005	0.0005	0.0008	0.0009

ND = not detected (<0.0005mg/kg)

NA = not applicable as the ¹⁴C-label is not present in this ring of the molecule of the test substance

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Figure B.7. 2: Proposed metabolic pathway of bensulfuron methyl in Japonica rice plants**B.7.1.2 Overall conclusions on nature of the plant residues**

A rice plant metabolism study was conducted using an application rate of 90 g a.s./ha ^{14}C bensulfuron methyl, approximately 1.5 times the maximum total annual GAP use rate. Two different forms of ^{14}C bensulfuron methyl were tested.

The total radioactive residue was not more than 0.009 mg/kg in the rice foliage (expressed as equivalent μg of bensulfuron methyl per gram of sample) at any sampling time (30, 60, 90 and 99 days after treatment). The concentration in rice grain at harvest was 0.003 mg/kg.

The primary metabolic pathway of bensulfuron methyl in Japonica rice plants is *O*-demethylation of the methoxypyrimidine moiety to yield *O*-desmethyl bensulfuron methyl (IN-F7880). Bensulfuron methyl also undergoes cleavage of the sulfonylurea linkage to yield sulfonamide (IN-N5297) and pyrimidine amine (IN-J0290). The sulfonamide cyclises to yield homo-saccharin (IN-B6895) (Figure B.7. 2).

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex IIIA 8.1)

According to the Working Document 7039/VI/95 EN (22/7/1997) metabolism studies in livestock are required when pesticide use may lead to significant residues (generally considered to be >0.1 mg/kg diet/day) in livestock feed. Based on the data from acceptable residue trials (Table B.7. 30), the dietary burden for animals (chicken, cattle and pig) was calculated to be below 0.1 mg/kg total diet (Table B.7. 5).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 5: Dietary burden calculations for chicken, beef and dairy cattle, pig

Crop group	Crop	% diet	DM Kg/ animal/day	% DM	Intake fresh Kg/ animal/day	Residue mg/kg	Residue intake mg/ animal/d	Dietary burden mg/kg diet/day
Chicken 0.12 Kg dry matter/day								
II Grains	Grains except maize	70	0.084	86	0.10	0.02	0.002	0.02
Total							0.002	0.02
Dairy Cattle 20 Kg dry matter/day								
II Grains	Grains except maize	40	8	86	9.30	0.02	0.19	0.009
III Straw	straw	20	4	86	4.65	0.1	0.47	0.023
Total							0.651	0.033
Beef Cattle 15 Kg dry matter/day								
II Grains	Grains except maize	80	12	86	13.95	0.02	0.28	0.02
Total							0.28	0.02
Pig 3 Kg dry matter/day								
II Grains	Grains except maize	80	2.4	86	2.79	0.02	0.06	0.019
Total							0.06	0.019

Although not necessary, livestock metabolism studies were conducted and reported below as additional information. In particular, metabolism of bensulfuron methyl in livestock was conducted with lactating goats and laying hens. In the lactating goat study, only the [phenyl(U)-¹⁴C]bensulfuron methyl metabolism was examined. In the laying hens study, both [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl metabolism was examined.

Characterisation of significant ¹⁴C residues and the elucidation of the metabolic pathway from the analysis of animal excreta were carried out.

B.7.2.1 Goat metabolism study

- 2) *Metabolism of [phenyl(U)-¹⁴C]DPX-F5384 in a lactating goat. Hunt, O.R., 1984; E.I. du Pont de Nemours and Company, Inc., Agricultural Products Department, Research and Development Division, Experimental Station Wilmington, Delaware 19898; unpublished report No.: AMR-219-84 of January 29, 1985. Date and location of study: April 24, 1984 – January 29, 1985; Enviro-Bio-Tech Ltd., Bernville, PA.*

Cross reference IIA, 6.2./01

Guidelines:

U.S. EPA Pesticide Assessment Guidelines Subdivision O, Series 171-4

GLP:

No

The study is acceptable as additional information

Materials and Methods:

Test Substance: ¹⁴C-DPX-F5384 technical

Labelled bensulfuron: [Phenyl(U)-¹⁴C] HOTC number not specified, specific activity: 15.0 µCi/mg, radiochemical purity: >99%. Non -radiolabelled bensulfuron: batch no. not specified, purity 99.5%.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

An acetone solution (0.5 mL) containing 3.22 mg (48.3 $\mu\text{Ci}/\text{mg}$) ^{14}C - labeled and 47 mg of unlabeled bensulfuron methyl were placed into each capsule containing goat feed. The solvent was evaporated. Thus, the amount of the test substance in each capsule was approximately 50.2 mg.

Dosing regime: One healthy lactating nanny goat was purchased obtained from Enviro-Bio-Tech Ltd., Bernville, PA. The animal was approximately 4 years old and had been in lactation 30-35 days at the time of the study. The goat weighed 100lbs at the start of the study. The animal was acclimatised for 6 days in an unheated barn and 2 days in a metabolism cage, prior to dosing. A daily single oral dose of one capsule, equivalent to a dietary burden of about 50-mg/kg in the diet, was administered to the lactating goat for seven consecutive days following the morning milking. Based on the daily supplementary feed of 1.0 kg/day, the dosing level was approximately 50 mg bensulfuron methyl/kg feed. The supplementary diet consisted of alfalfa and hay. The test goat was also fed with an additional diet of 0.34 to 0.79 kg (0.75 to 1.75 lb) hay.

Sampling: Urine, faeces, and milk were collected daily over the dosing period and sub-samples were analysed for total radioactivity by liquid scintillation counting (LSC). Milk, collected from the animal in the evening and the next morning following the first dose, was combined and labeled as the Day 1 sample. Subsequent daily milk samples (2 through 7) were collected in a similar manner. Control milk, urine and faeces were collected on the day immediately prior to dosing. The animal was sacrificed within 26 hours after final dosing (7 days after initial dose). Blood, brain, heart, kidney, liver, pancreas, spleen, muscle samples (flank, loin and leg) and fat samples (back, omental, peripheral and renal), were collected for analysis. All samples were frozen immediately, shipped frozen to the experimental station and stored at -20°C .

Extraction of samples:

A 500 g portion of the liver was freeze-dried and homogenised, extracted sequentially with n-hexane (2 x 400 mL), methylene chloride:methanol:2 M ammonium carbonate (MMA) (3:4:1, v/v/v; 3 x 250 mL), and 0.1N sodium hydroxide (3 x 250 mL). The hexane extract was discarded, as no detectable radioactivity was present in it. The organic phase was separated from the MMA extract and the pH of the aqueous phase was adjusted to 5 using dilute phosphoric acid. The aqueous phase was partitioned with methylene chloride (3 x 250 mL) followed by methylene chloride:2-butanone (3:1, v/v, 3 x 250 mL). The 0.1 N sodium hydroxide extract was lyophilised and the radioactive residues in the solids were extracted using methanol. All the extracts containing radioactivity were evaporated to 1-2 mL under reduced pressure.

A 750 mL portion of three different milk samples was extracted separately with n-hexane. Then an equal portion of methanol was added to each milk sample to precipitate protein. Solid material from each sample was removed by centrifugation and the methanol was removed by evaporation under reduced pressure. The aqueous phase was extracted with chloroform. The chloroform was removed by evaporation under reduced pressure followed by a stream of nitrogen until an oily material remained. The radioactive residues were extracted from the oil into methanol.

Urine samples (25 mL aliquots) on Days 1, 3, 5, and 7 were extracted separately. Each urine sample was mixed with 25 mL of water, adjusted to pH 4 with dilute (1:5, v/v) phosphoric acid, and extracted with n-hexane (2 x 50 mL) followed by methylene chloride (4 x 50 mL). Then the pH of each aqueous phase was adjusted to pH 1.5 with concentrated hydrochloric acid (HCl) and extracted using methylene chloride:2-butanone (3:1, v/v; 3 x 50 mL). The same solvent extracts were pooled and radioactivity determined by LSC. The extracts containing radioactive residues were concentrated to 1-2 mL under reduced pressure followed by a stream of nitrogen.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Day 1, 4, and 7 faeces (75-100 g) samples were freeze-dried, extracted sequentially with n-hexane (2 x 100 mL), methylene chloride (4 x 100 mL), and methylene chloride:methanol (1:1, v/v, 2 x 100 mL). Methylene chloride and methylene chloride:methanol extracts were evaporated separately to dryness under reduced pressure, reconstituted in methanol and assayed by LSC.

Determination of total radioactivity: Total radioactive residues (TRR) in liquid samples were determined by liquid scintillation counting (LSC). The TRR in the tissues were determined by combustion of sub-samples followed by LSC. Tissues with significant residues (>0.3 mg/kg) were exhaustively extracted. The Limit of Detection of the analysis method was 0.005mg/kg in milk samples and 0.3 mg/kg in tissue and organ samples.

Chromatography of extracts: Concentrated extracts from liver, milk, urine and faeces were analysed by thin-layer chromatography (TLC) using two different methods. The first method consisted of 0.25 mm pre-adsorbed plates (Whatman LK6F[®]) and a solvent system of methylene chloride:methanol:ammonium hydroxide.(150:50:3,v/v/v). Aliquots (50 to 100 µL) of processed extracts from the milk, liver, urine, and faeces were spotted and developed for 15 cm using the first method.

The second system consisted of a two phase 0.25 mm TLC plates (Whatman CS5[®]) and a solvent system of methylene chloride:acetonitrile:water:acetic acid (150:23:3:2.7, v/v/v/v). The TLC plates were developed for 1 cm in reverse phase section followed by 14 cm of silica gel GF using the second method. Radiolabeled metabolites were identified by co-chromatography with authentic reference standards and R_f values were compared with identical systems used with the bensulfuron methyl rat metabolism study.

Findings:

Animal Observations: There was no record of signs of adverse effects which could be attributed to the administration of bensulfuron methyl. The milk yield gradually decreased from 2700 mL on Day 1 to 1440 mL on Day 7, which could be attributed to stress in the metabolism cage.

Distribution of radiolabeled residues

All the radioactive residues in milk and liver are expressed as mg equivalents of bensulfuron methyl/kg of the corresponding sample. The urine ¹⁴C residues were expressed as % of the TRR in the urine for the sampling day.

Excreta: The administered radioactivity was excreted quantitatively. Faecal and urinary excretion was almost equal with 49% in the faeces and 52% accounted in the urine (Table B.7. 6).

Milk: Low levels of radioactive residue were detected in the whole milk. The maximum level of total radioactive residues (TRR) in milk was 0.031 mg/kg (detection limit 0.005 mg/kg). A plateau (0.027-0.031 mg/kg) in the milk was established beginning with the Day 4 sample. The TRR in the milk samples were <0.07% of the total administered dose (Table B.7. 7).

Total radioactive residues in organs and tissues: Total radioactive residues in the various organs and tissues, except liver, were below the limit of detection (<0.3 mg/kg equivalent of bensulfuron methyl) and accounted for only ca. <0.5% of the administered radioactivity (Table B.7. 8). The total radioactive residues in the liver were 0.4 mg/kg or approximately 0.07% of the total administered dose.

Material balance: The overall recovery of the administered radioactivity through faeces, urine, and organs and tissues was 101% (Table B.7. 8).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 6: Elimination of the radioactivity in the lactating goat following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet

Time (day)	% Total administered radioactivity		
	Urine	Faeces	Total
Day 1	6.26	5.99	12.25
Day 2	6.20	7.13	13.23
Day 3	2.98	7.72	10.70
Day 4	6.20	5.00	11.20
Day 5	9.24	7.44	16.68
Day 6	9.95	6.94	16.89
Day 7	11.56	8.33	19.89
Total	52.4	48.55	100.99

Table B.7. 7: Summary of total radiolabeled residues in milk at various sampling times following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet

Sample time (day no.)	Concentration ^a , mg/kg
Control	ND ^b
Day 1	0.018
Day 2	0.025
Day 3	0.018
Day 4	0.027
Day 5	0.028
Day 6	0.031
Day 7	0.026

^a Concentration of radioactive residues were expressed as mg/kg of milk equivalents of bensulfuron methyl

^b ND = non detectable; limit of detection = 0.005 mg/kg

Table B.7. 8: Summary of total radiolabeled residues in organs and tissues collected at sacrifice following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Matrix/tissue	Concentration ^a mg/kg	% Dose
Whole blood	ND ^b	<0.01
Brain	ND	<0.01
Heart	ND	<0.01
Kidney	ND	<0.01
Liver	0.40	0.07
Pancreas	ND	<0.01
Spleen	ND	<0.01
Flank muscle	ND	NA ^c
Leg muscle	ND	NA
Loin muscle	ND	NA
Back fat	ND	NA
Omental fat	ND	NA
Peripheral fat	ND	NA
Renal fat	ND	NA

^a Concentration of radioactive residues were expressed as mg/kg of milk (mg/kg) equivalents of bensulfuron methyl

^b ND = non detectable; limit of detection = 0.3 mg/kg

^c NA = not applicable– total weight unavailable

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Characterisation of residues: Detailed data on the characterization /identification of the radioactive residues are provided in Table B.7. 12 and Table B.7. 13.

Urine: Almost half of the administered radioactivity (*ca.* 52%) was recovered in the urine. Total extraction efficiencies in the urine were 88 to 90% TRR. Less than 0.1% TRR was extracted into n-hexane. Methylene chloride and methylene chloride:2-butanone (3:1, v/v, pH 1.5) extracted 46-77% and 17-43%, respectively (Table B.7. 9). *O*-Desmethyl bensulfuron methyl (IN-F7880, 21-43% TRR) was the major identified metabolite. Other minor metabolites present in the urine were 4-hydroxypyrimidine bensulfuron methyl (IN-N8989, 0.3-1.0%), homosaccharin (IN-B6895, 1.1-2.3%) and sulphonamide (IN-N5297, 0.2-0.8% TRR). The total unknown metabolites were in a range of 38.2 to 55.5% of TRR (GU1 2.6-4.2% and GU2 (34-53%) in the urine (Table B.7. 12).

Table B.7. 9: Summary of extraction of radioactivity from the goat urine following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Urine sample collection day	% TRR extracted				
	n-hexane	Methylene chloride ^a	Methylene chloride ^b	Methylene chloride: 2-butanone ^c	Remained in urine
Day 1	0.2	72.3	17.0	2.4	8.1
Day 3	0.3	45.7	42.7	1.9	9.4
Day 5	0.4	77.4	12.0	2.2	7.9
Day 7	0.1	69.0	18.8	2.4	9.7

^a Urine at pH 4 was partitioned with methylene chloride

^b Urine at pH 1.5 was partitioned with methylene chloride

^c Urine at pH 1.5 was partitioned with methylene chloride:2-butanone (3:1, v/v).

Faeces: Extraction of faecal samples (Day 3, Day 5, and Day 7) separately with hexane, methylene chloride, and methylene chloride: methanol mixture (1:1, v/v) removed 50-60% of the TRR (Table B.7. 10). The faecal metabolites were identified by co-chromatography using TLC methods. *O*-Desmethyl bensulfuron methyl (IN-F7880, 16-20% TRR) 4-hydroxypyrimidine bensulfuron methyl (7-12%), homosaccharin (IN-B6895, 0.04-2.4%) and sulphonamide (2.4-9.1%) were the compounds identified. Two unknown metabolites, GF1 (0-2.6%) and GF2 (2.1-4.6%) were also detected. The composition of the radioactive residues in the faeces is shown in Table B.7. 12.

Muscle and kidney: The TRR was below the limit of detection (<0.3 mg/kg) in the muscle and kidney samples (Figure B.7. 3). Therefore, no further characterisation of the radioactive residues in the muscle and kidney tissues was carried out.

Table B.7. 10: Summary of extraction of radioactivity from faeces of the goat following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Faeces sample collection day	n-hexane	Methylene chloride	Methylene chloride: Methanol (1:1, v/v)	Total extracted	Un-extracted
Day 1	0.2	28.7	27.3	56.2	43.9
Day 4	0.4	21.4	26.6	48.4	51.6
Day 7	0.2	22.5	36.6	59.3	40.6

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 11: Summary of extraction of radioactivity as percent of TRR from liver of the goat following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Solvent	Liver	Milk		
		Day-4	Day-5	Day-7
n-Hexane		9.6	4.3	<0.1
Methylene chloride	28.6	75.1	86.5	87.0
Methylene chloride:methanol-2 M (NH ₄) ₂ CO ₃	39.8	NA	NA	NA
0.1 N Sodium hydroxide	19.3	NA	NA	NA
Total extracted	86.0	84.7	90.8	87.0
Total unextracted	14.0	16.2	9.4	13.0
Total recovered	100.0	100.9	100.2	100.0

Table B.7. 12: Characterisation and identification of recovered radiolabel in urine and faecal excreta of goat following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Analyte	% TRR of each day samples					
	Urine			Faeces		
	Day 3	Day 5	Day 7	Day 1	Day 4	Day 7
Extractable:				56.2	48.4	59.3
Bensulfuron methyl	0.3	0.1	0.2	18.0	9.1	4.4
IN-F7880	43.0	21.1	22.3	15.6	18.9	20.3
IN-N8989	1.0	0.3	0.6	11.4	6.7	12.4
IN- B6895	1.1	1.7	2.3	0.4	2.4	1.2
IN-N5297	0.8	0.6	2.0	7.0	2.4	9.1
Unknown 1	3.8	4.2	2.6	1.1	ND	2.6
Unknown 2	34.4	48.8	52.9	4.6	5.7	2.1
Total extractable	84.4	76.8	82.9	58.1	45.2	52.1
Unextractable	9.4	7.9	9.7	43.9	51.6	40.6
Total recovered	93.8	84.7	92.6	102.0	96.8	92.7

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 13: Characterisation and identification of extracted recovered radiolabel from milk and liver of goat following the administration of a daily single oral dose with [phenyl(U)-¹⁴C]bensulfuron methyl equivalent to 50 mg/kg in the diet for seven consecutive days

Analyte	mg/kg ^a (% TRR)			
	Milk			Liver
	Day 4	Day 5	Day 7	
Extractable^a:	75.1			
Bensulfuron methyl	0.003 (10.0)	0.002 (8.0)	0.002 (7.0)	ND
IN-F7880	0.007 (27.0)	0.013 (46.0)	0.009 (36.0)	0.26 (64.0)
IN-N8989	0.004 (15.0)	0.002 (6.0)	0.009 (33.0)	
IN- B6895	0.007 (26.0)	<0.001 (<3.0)	<0.001 (<3.0)	0.008 (2.0)
IN-N5297	0.003 (11.0)	0.003 (12.0)	0.003 (11.0)	0.006 (1.5)
Unknown 1	<0.001 (<3.0)	0.002 (8.0)	0.002 (8.0)	0.05 (13.0)
Unknown 2				ND
Total extractable	84.7	90.8	87.0	
Unextractable	16.2	9.4	13.0	
Total recovered	100.9	100.2	100.0	

^aThe amounts of the radioactive residues in the milk and liver samples were expressed as the % of the TRR in the original report. These residues were calculated as mg/kg of the sample for this dossier.

ND = not detected (<0.005 mg/kg)

Milk: Analyses of milk from Day 4, Day 5, and Day 7 indicated the presence of six metabolites together with a trace amount of bensulfuron methyl (≤ 0.002 mg/kg). *O*-Desmethyl bensulfuron methyl (IN-F7880) was the major metabolite and was found at 0.013 mg/kg (46% TRR) in Day 5 milk. The second major metabolite was pyrimidine ring hydroxylated bensulfuron methyl (IN-N8989, 0.009 mg/kg, 33% TRR). Sulphonamide (IN-N5297, 0.003 mg/kg, 11%TRR) and homosaccharin (IN-B6895, 0.007 g/kg, 26% TRR in Day 4 milk only) were also observed. Two unknown metabolites, GMU1 (3-8% TRR, 0.002 mg/kg) and GMU2 (5-20% TRR, 0.005 mg/kg) were also detected.

Liver: The methylene chloride, methylene chloride: methanol: 2 M ammonium carbonate (3:4:1, v/v/v), and 0.1 N sodium hydroxide extracts of the liver sample contained 28.6, 39.8, and 19.3% TRR. Unextracted residues accounted for 14% TRR.

Bensulfuron methyl was not detected in the liver. *O*-Desmethyl bensulfuron methyl (IN-F7880) was the principle metabolite accounting for 64% of the radioactivity in the extracts. Other minor components were homosaccharin (IN-B6895) and sulphonamide (IN-N5297). One unknown metabolite, GLU1 (13% TRR) was also detected.

Conclusion:

A daily single oral dose of ¹⁴C-bensulfuron methyl equivalent of 50-mg/kg in the diet was administered to a lactating goat for seven consecutive days. The administered radioactivity was rapidly excreted from the lactating goat. Low levels of radioactivity were found in the milk (0.018-0.031 mg/kg), which reached a plateau at around 5 days after the first dose. Liver was the only tissue containing detectable radioactive residues. The radioactive residue level (0.4 mg/kg) was only slightly above the detection limit (0.3 mg/mL) (calculated or equivalent bensulfuron methyl). Bensulfuron methyl is almost completely metabolised. Bensulfuron methyl was not detected in the liver sample. Milk samples contained a small amount (≤ 0.003 mg/kg) of parent bensulfuron methyl.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

The principle metabolites were *O*-desmethyl bensulfuron methyl (IN-F7880) and hydroxypyrimidinyl bensulfuron methyl (IN-N8989). Homo-saccharin (IN-B6895) and sulphonamide (IN-N5297) were also present in the urine, faeces and liver samples.

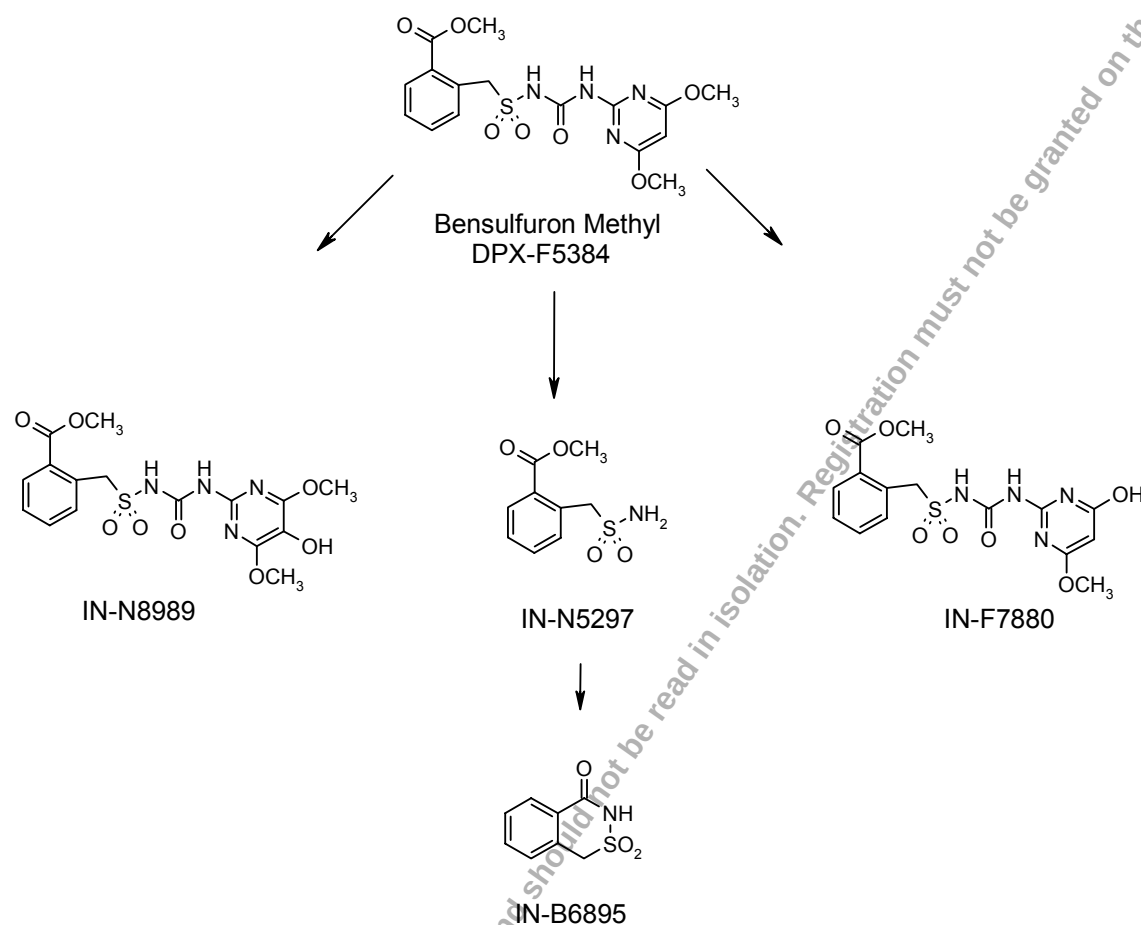
Metabolic pathway: The primary metabolic pathway included hydroxylation and *O*-demethylation yielding IN-N8989 and IN-F7880, respectively. The minor pathway included cleavage of sulfonylurea bridge to form sulphonamide (IN-5297), which then forms homosaccharin (IN-B6895).

B.7.2.1.1 Nature of residues in goat

There was minimal transfer of ^{14}C bensulfuron methyl equivalent residues to the milk and tissues following 7 consecutive days of dosing. ^{14}C bensulfuron methyl and its metabolites were rapidly excreted from the lactating goat. Essentially all administered radioactivity was eliminated in the urine (~52%) and faeces (~49%). Total radioactive residues (mg bensulfuron methyl equivalents) from the [phenyl(U)- ^{14}C]bensulfuron methyl treated goat were negligible. No major tissues, except liver (0.4 mg/kg), had detectable ^{14}C residues. Most of the liver residue (86%) was solvent extractable. Low levels of ^{14}C residues were found in milk (0.018-0.031 mg/kg). Most of the milk residues (between 85 and 95%) were solvent extractable. Parent, bensulfuron methyl, was present at trace levels in the milk (<0.01 mg/kg), but was not detected in the liver. Metabolites in the milk and liver were consistent with those found in both the urine and faecal extracts. The principle metabolites in the goat were *O*-desmethyl bensulfuron methyl, 4-hydroxypyrimidine bensulfuron methyl.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Figure B.7. 3: Proposed metabolic pathway of bensulfuron methyl in the goat**B.7.2.2 Laying hen metabolism study**

- 3) *Metabolism study of ^{14}C bensulfuron methyl in laying hens. Scott, M.T., 1991; unpublished report No.: AMR-1454-89 of January 17, 1991. E.I. du Pont de Nemours and Company, Inc., Agricultural Products Department, Research and Development Division, Experimental Station Wilmington, Delaware 19880-0402; Hazleton Laboratories America, Inc., Madison, Wisconsin; Date of study: April 24, 1989 – January 17, 1991.*

Cross reference IIA, 6.2./02

Guidelines:

U.S. EPA Pesticide Assessment Guidelines Subdivision O, Series 171-4 (a)(3)

Deviations: none

GLP:

Experimental work performed before October 16, 1989 was conducted in the spirit of GLP, and experimental work performed after October 16, 1989 was conducted in compliance with EPA Good Laboratory Practice Standards (40 CFR Part 160).

The study is acceptable as additional information.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Materials and Methods:

Test Substance: ^{14}C -DPX-F5384 technical

Labelled bensulfuron: [Phenyl(U)- ^{14}C] batch no.HOTC320, radiochemical purity >98%; [Pyrimidine-2- ^{14}C] batch no.HOTC319, radiochemical purity >97%

Non-radiolabelled bensulfuron: batch No. not specified, purity 99.5%.

The metabolism of bensulfuron methyl was investigated in young laying hens (single-comb, "White Leghorn", 34 weeks old, body weight range 1.2–1.7 kg). The birds were obtained from a local supplier and were housed individually in laying cages; each treatment group was housed in a separate deck to prevent cross contamination.

Two radiolabeled forms of bensulfuron methyl were used: (1) [phenyl(U)- ^{14}C]bensulfuron methyl (Batch No. HOTC #320; radiochemical purity 98.0%) and (2) [pyrimidine-2- ^{14}C]bensulfuron methyl (Batch No. HOTC #319; radiochemical purity >97%). Each radiolabeled test substance was diluted with bensulfuron methyl analytical standard (purity, 99.5%) to a final specific activity of about 4.77 $\mu\text{Ci/mg}$ and 4.42 $\mu\text{Ci/mg}$ for [phenyl(U)- ^{14}C]bensulfuron methyl and [pyrimidine-2- ^{14}C]bensulfuron methyl, respectively. Dose capsules were prepared using [phenyl(U)- ^{14}C]bensulfuron methyl (0.013 mg/capsule) and [pyrimidine-2- ^{14}C]bensulfuron methyl (0.013 mg/capsule). Additional dose capsules for each radiolabel were prepared separately using 6.0 mg/capsule for administering the high dose group hens.

Dosing Regime: This study included four treatment groups and a control group. Each treatment group had five hens and 2 hens were in the control group. Two treatment groups were maintained for each radiolabeled test substance: low dose (0.013 mg/dose, 0.1 mg/kg dietary, the proposed tolerance) and high dose groups (6.0 mg/dose, 50 mg/kg dietary, 500 times the tolerance). The objective of dosing at 500 times the tolerance was for generation of suitable quantities of metabolites for identification by LC-MS analysis. A daily single oral dose was administered to each hen with either [phenyl(U)- ^{14}C]bensulfuron methyl or [pyrimidine-2- ^{14}C]bensulfuron methyl at the respective dose level. Eggs and excreta were collected prior to the first dose. Subsequent doses were administered after the morning's collection of each day's excreta and egg samples. Test substances were administered to the low dose group hens for 14 consecutive days (Days 0-13) and to the high dose hens for 5 days (Days 0-4). The control hens were not dosed.

Sampling:

Daily egg production and excreta were collected once each day. Egg production during the study had a mean value of one egg/day for the treated groups. Samples were pooled according to day, sample matrix and treatment group and then weighed. Animals were sacrificed approximately 6 hours after final dosing. Fat, skin with fat, kidneys, liver, and breast and thigh muscle were collected. Blood was refrigerated until analysis was complete and then stored frozen. All other samples were stored below 0 $^{\circ}\text{C}$ before and after analysis.

Determination of total radioactive residues and extraction and analysis:

Total radioactive residues in tissues were determined by combustion of subsamples followed by liquid scintillation counting (LSC). Eggs skin with fat, fat, liver, breast muscle, and thigh muscle from the low dose group had radioactive residues below the limit of detection (LOD, 0.002 mg/kg) therefore, excreta samples were used to identify metabolites. Day-3 and Day-4 lyophilised excreta samples from the high dose groups were extracted with n-hexane at 40 $^{\circ}\text{C}$ for 30 minutes using a sonication bath, centrifuged, decanted, and the extraction procedure repeated. The solids were further extracted three times with methylene chloride: methanol: 2 M ammonium carbonate (3:4:1, v/v/v) using the same sonication method. The same solvent extracts were pooled for each sample separately and

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

concentrated by rotary evaporation. The concentrated extracts were adjusted to pH 4-5 using formic acid, centrifuged, filtered through a 0.45 µm membrane, and the supernatant samples analysed by LSC and HPLC. Several HPLC methods were used for the isolation and identification of metabolites. Preparative HPLC was conducted using a Rainin Dynamax® Cyano column and analytical methods used a DuPont Zorbax® Cyano column. The solvent gradient system for the analytical method was mixtures of acetonitrile and water containing triethyl amine (TEA) and phosphoric acid. The pump A used acetonitrile:water (9:1, v/v) containing 0.1% (v/v) TEA and phosphoric acid. The pump B used water containing 0.1% (v/v) TEA buffered with phosphoric acid to pH 2.5. The preparative methods used the same solvent mixtures at different ratios. Isolated metabolites were analysed by packed capillary column liquid chromatography/mass spectrometry using a Finnigan MAT 8230 magnetic mass spectrometer. A continuous flow probe was used for fast atom bombardment (FAB) mass spectra using glycerol (5-10%, v/v) in mobile phase (acetonitrile : water to pH 3 with formic acid).

Findings:

Animal health: Treated hens were in good health and did not display signs of adverse effects that could be attributed to the administration of bensulfuron methyl. No significant weight loss or changes in egg production pattern were recorded.

Table B.7. 14: Elimination of the total administered radioactivity in low dose and high dose groups laying hens following a daily single oral dose with either [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl

Time (days)	Percent of total administered radioactivity in excreta ^a			
	Low dose group hens (0.1 mg/kg diet)		High dose group hens (50 mg/kg diet)	
	[Phenyl(U)- ¹⁴ C]	[Pyrimidine-2- ¹⁴ C]	[Phenyl(U)- ¹⁴ C]	[Pyrimidine-2- ¹⁴ C]
0	0.08	ND	ND	ND
1	4.61	5.50	12.9	15.3
2	6.35	6.24	15.4	28.1
3	5.58	5.81	18.4	14.9
4	6.29	6.10	16.8	19.0
5	5.40	5.81	9.79 ^a	13.4 ^a
6	5.42	6.00	NA	NA
7	5.73	6.14	NA	NA
8	5.96	6.11	NA	NA
9	6.26	6.29	NA	NA
10	6.02	6.7	NA	NA
11	6.43	6.23	NA	NA
12	6.01	5.97	NA	NA
13	5.84	5.89	NA	NA
13 ^a	3.29	2.65	NA	NA
GI tract	2.53	3.24	7.12	5.92
Pan paper	3.36	2.88	1.59	0.96
Total	85.1	87.5	82.0	87.6

^a Six hours past the last dose

Dose administration: The dose levels were approximately 0.1 mg/kg (low dose) and 50 mg/kg (high dose) of bensulfuron methyl in the daily diet for the low dose groups and high dose groups of hens, respectively. The low dose was chosen, as it was the proposed tolerance. There was no reasonable anticipation of occurrence of bensulfuron methyl in poultry feed items.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Distribution of radiolabeled residues

Overall recovery ranged from 82.8 to 88.3%.

Excreta: For all the treated groups, radioactivity recovery ranged from 82.8 to 88.3% of the total administered dose, with the majority (over 73%) eliminated in the excreta. Elimination of radioactivity was relatively fast and became constant approximately 48 hours after administration of the initial dose (Table B.7. 14).

Eggs: For the low dose groups, the radioactive residues in the eggs were below the limit of detection (0.002 mg/kg). Even high dose group eggs contained <0.01% of the total dose.

Total tissue residues and material balance: Total ^{14}C residues in the blood, fat, and various organs and tissues from the low dose group hens were <0.002 mg/kg (LOD). The liver samples from the high dose hens contained 0.09% of the total dose in samples from both the [phenyl(U)- ^{14}C] and [pyrimidine-2- ^{14}C]labeled groups.

Characterisation of residues:

The lack of residues (<0.002 mg/kg) in the eggs, skin with fat, fat, liver, breast muscle, and thigh muscle from the low dose group hens precluded isolation and identification of any radioactive residues.

Excreta: Day 3 and Day 4 excreta from the high dose group hens were used for the characterisation of the radioactive residues. The parent bensulfuron methyl and major metabolites were isolated by preparative HPLC. Unmetabolised bensulfuron methyl was the major component in the excreta extracts. Chromatographic profiles of excreta extracts from [phenyl(U)- ^{14}C] and [pyrimidine-2- ^{14}C] dosed birds were similar, indicating that excreta samples contained negligible concentration of polar sulfonylurea bridge cleavage products. *O*-Desmethyl bensulfuron methyl (Metabolite I, IN-F7880) was the major metabolite in the excreta. Metabolite I (IN-F7880) undergoes pyrimidine ring opening to yield Metabolite II, which undergoes cyclisation with an elimination of a water molecule to yield dehydrated Metabolite II. The Metabolite II also undergoes a loss of carboxyl group to yield Metabolite III (IN-78184).

Hydroxylation of the phenyl ring was the next major metabolic route in hens. The phenyl ring hydroxylated metabolites were isolated from the excreta extracts of the [pyrimidine-2- ^{14}C]bensulfuron methyl-dosed hens and identified by the LC-MS. While the exact position of the hydroxyl group was not clear from the mass spectral fragmentation, this metabolite was tentatively identified as IN-W8956, as the 4-position of the phenyl ring is preferred for the oxidation reaction.

Metabolic pathway: Two primary metabolic pathways were observed (Figure B.7. 4). The first pathway involved the *O*-demethylation to yield *O*-desmethyl bensulfuron methyl (Metabolite I, IN-F7880). Metabolite I (IN-F7880) undergoes pyrimidine ring opening to yield Metabolite II, which undergoes cyclisation with an elimination of a water molecule to yield dehydrated Metabolite II. The Metabolite II also undergoes a loss of carboxyl group to yield Metabolite III (IN-78184). Heterocyclic ring opening products of *O*-desmethyl bensulfuron methyl (IN-F7880) were observed in the excreta of 50 mg/kg dose group hens. The second metabolic pathway involved hydroxylation of the phenyl ring to yield IN-W8956.

Conclusion:

Minimal transfer of bensulfuron methyl equivalent residues (<0.002 mg/kg) to the egg, skin with fat, fat, liver, and muscle was observed following daily single oral dosing at a level equivalent to *ca.* 0.013-mg/kg for 14 consecutive days. Even in the high dose group (6 mg/dose, 500 times the tolerance) the residues were 0.01 to 0.1% of the total administered dose in eggs and edible tissues.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

For both the treated groups, radioactivity mass balance was in the range from 82.8 to 88.3% of the total administered dose, with the majority (over 73%) eliminated in the excreta. Metabolites were identified from the high dose group hen excreta.

The metabolic profile in the excreta samples from the [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl dosed hens was similar. In addition to unmetabolised bensulfuron methyl, IN-7880 (Metabolite I), IN-W8956, Metabolite II, dehydrated Metabolite II, and IN-78184 (Metabolite III) were identified in the excreta from high dose group hens.

The metabolic fate of bensulfuron methyl in the laying hen was consistent with bensulfuron methyl metabolism in the laboratory rat and lactating goat.

Table B.7. 15: Level of radioactive residues in eggs from low dose and high dose group hens following a daily single oral dose with either [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl

Time (days)	Low dose group hens (0.1 mg/kg diet)		High dose group hens (50 mg/kg diet)	
	[Phenyl(U)- ¹⁴ C] mg/kg	[Pyrimidine-2- ¹⁴ C] mg/kg	[Phenyl(U)- ¹⁴ C] mg/kg	[Pyrimidine-2- ¹⁴ C] mg/kg
0	<0.001 ^a	<0.001 ^a	ND	ND
1	ND	ND	0.013	ND
2	ND	ND	0.038	0.063
3	ND	ND	0.042	0.028
4	ND	ND	0.052	0.017
5	<0.001	ND	0.023	0.062
6	ND	ND	NA	NA
7	ND	ND	NA	NA
8	ND	ND	NA	NA
9	ND	ND	NA	NA
10	ND	ND	NA	NA
11	ND	ND	NA	NA
12	<0.001	ND	NA	NA
13 ^b	ND	ND	NA	NA
13 ^a	ND	ND	NA	NA

^a Possible contamination, not included in total radioactive residues

^b Six hours past the last dose (sacrifice day)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 16: Level of radioactive residues in tissues from low dose and high dose group hens following a daily single oral dose with either [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl

Samples	Low dose group hens (0.1 mg/kg diet)		High dose group hens (50 mg/kg diet)	
	[Phenyl(U)- ¹⁴ C] mg/kg	[Pyrimidine-2- ¹⁴ C] mg/kg	[Phenyl(U)- ¹⁴ C] mg/kg	[Pyrimidine-2- ¹⁴ C] mg/kg
Blood	ND	ND	0.203	0.17
Fat	ND	ND	ND	0.016
GI tract	0.012	0.014	3.067	2.265
Kidneys	ND	0.001	0.336	0.352
Liver ^b	<0.001	<0.001	0.678 ^b	0.588 ^b
Muscle (breast)	ND	ND	0.023	ND
Muscle (thigh)	ND	ND	0.023	0.024
Skin with fat	ND	ND	0.056	0.048

^a Possible contamination, not included in total radioactive residues

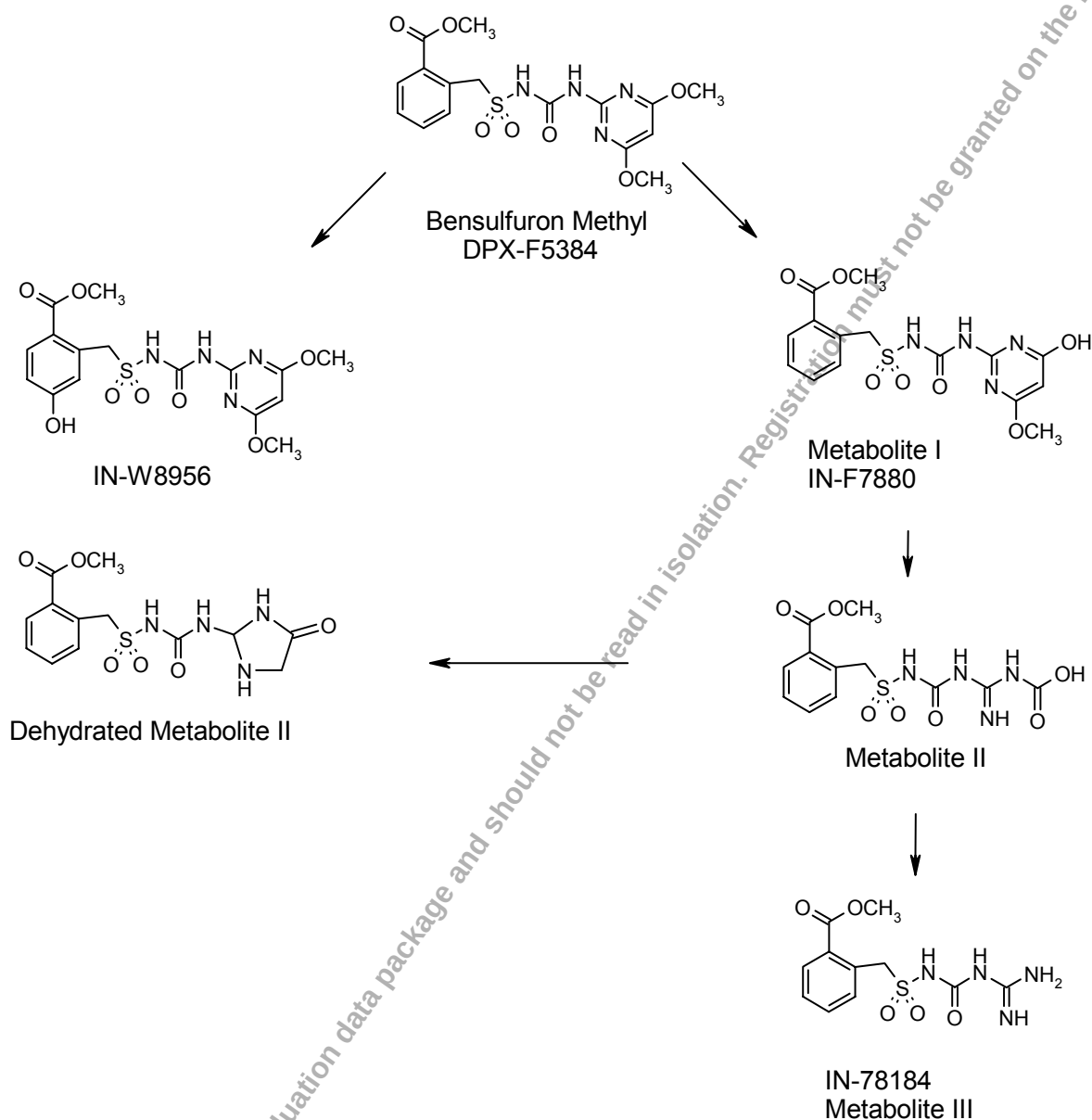
^b Total residues from hens, extrapolated to a dose of 10 mg/kg dietary, would have been 0.10-0.14 mg/kg. Based on the presence of numerous extractable faecal metabolites, it is reasonable to assume that concentrations of individual metabolites in liver would each be <0.05 mg/kg (bensulfuron methyl equivalents). Furthermore, the concentration of ring-opened metabolites or cleavage products would be even when corrected for individual molecular weights.

B.7.2.2.1 Nature of residues in hens

¹⁴C bensulfuron methyl and its metabolites were rapidly excreted from the laying hen. There was minimal transfer of ¹⁴C bensulfuron methyl equivalent residues to the eggs and tissues following 14 consecutive days of dosing. Total radioactive residues (mg bensulfuron methyl equivalents) from the [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl-treated hens was minimal. The ¹⁴C residues in eggs, blood, and all tissue samples, except the GI tract (0.012-0.014 mg/kg), were below the detection limit (0.002 mg/kg). No further characterisation of these samples was attempted because of the low ¹⁴C residues. The rapid excretion and lack of significant uptake in tissues and organs is similar to the results found in the lactating goat. Most of the administered radioactivity was eliminated in the excreta (approximately 80%). The major excreted metabolites included *O*-desmethyl bensulfuron methyl (IN-F7880), a metabolite found in both the rat and goat metabolism studies, phenyl ring hydroxylated bensulfuron methyl (IN-W8956), and three pyrimidine-ring opened sulfonylureas – Metabolite II, dehydrated Metabolite II, and Metabolite III. The latter metabolites were not observed in other animal studies. As no significant residues were found in eggs or tissues from poultry dosed at the tolerance level, extremely low concentration of these metabolites, if formed, should be of no regulatory concern.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Figure B.7. 4: Proposed metabolic pathway of bensulfuron methyl in laying hens**B.7.2.3 Overall conclusions on metabolism in animals**

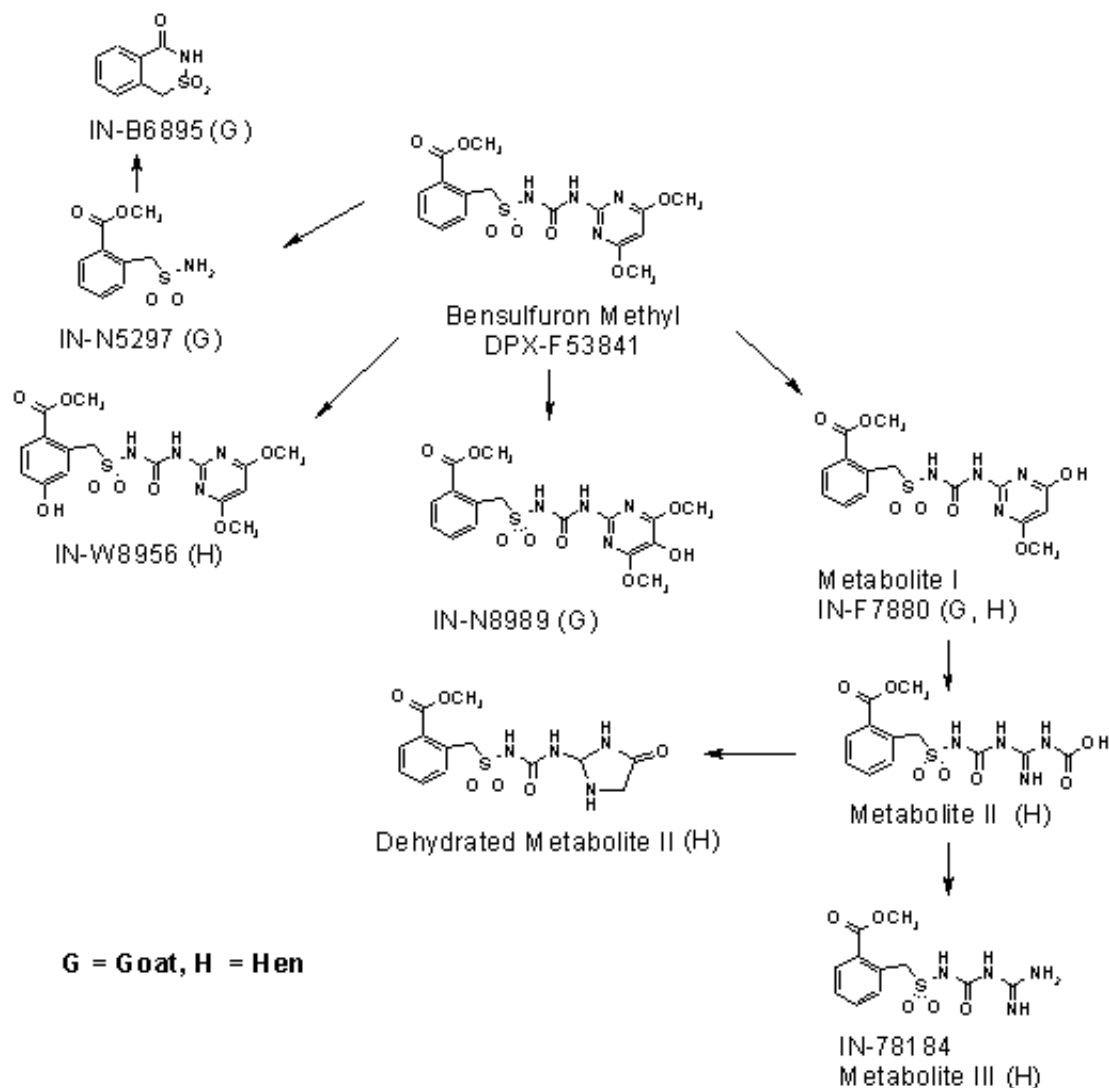
Although the dietary burden in livestock resulted below the trigger value, two studies on the metabolism in livestock were submitted: one in goat and one in laying hens. Despite the fact that only one ring was labeled in the goat study, a tentative pathway of bensulfuron-methyl in livestock was proposed. Most of the dosed radioactivity (greater than 73%) was rapidly excreted from the livestock (goat and poultry) within 48 hours of the administered dose. The major component in animal excreta was unchanged bensulfuron methyl. The primary pathway was *O*-desmethylation of the pyrimidine ring yielding *O*-desmethyl-bensulfuron methyl (IN-F7880). A second metabolic pathway involved hydroxylation of the phenyl ring to form IN-W8956. Another major metabolite was formed in the goat by hydroxylation of the pyrimidine ring to yield IN-N8989. A minor metabolic pathway in the goat involved cleavage of the sulfonylurea bridge to form the sulphonamide (IN-N5297). A minor pathway observed in the hen involved hydroxylation of the phenyl ring to yield IN-W8956.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

There was minimal potential for bioaccumulation of bensulfuron methyl in the tissues. The total ^{14}C residues found in the blood, fat, various tissues, and eggs in the hen were all less than 0.002 mg/kg. Similarly, the total ^{14}C residues found in various organs and tissues in the goat, with the exception of liver tissue, were all below the level of detection (0.3 mg/kg). The residue level found in the liver tissue was 0.4 mg/kg. The total ^{14}C residue in goat milk reached a plateau (0.027-0.031 mg/kg) in 4 days. Analysis of the milk indicated the presence of six metabolites together with a trace amount of bensulfuron methyl (≤ 0.002 mg/kg). *O*-Desmethyl bensulfuron methyl (IN-F7880) was the major milk metabolite and was found at 0.013 mg/kg (46% TRR) in Day 5 milk. The second major metabolite was pyrimidine ring hydroxylated bensulfuron methyl (IN-N8989, 0.009 mg/kg, 33% TRR). Sulphonamide (IN-N5297, 0.003 mg/kg, 11%TRR) and homosaccharin (IN-B6895, 0.007 g/kg, 26% TRR in Day-4 milk only) were also observed. The overall metabolic pathway of bensulfuron methyl in animals is proposed in Figure B.7. 5.

Figure B.7. 5: Proposed metabolic pathway of bensulfuron methyl in animals



Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

Proposed residue definition

Plant:

Based on the results of the metabolism study on rice plants and on the toxicological evaluation reported in B6, the definition of residue for monitoring and risk assessment purposes is proposed as bensulfuron methyl.

Animal:

Since the livestock dietary burden is below the trigger value of 0.1 mg/kg total diet, no metabolism studies are required. Additional information was provided for goats and hens. Based on this and on the toxicological evaluation reported in B6, the proposed residue definition for monitoring and risk assessment purposes can be given as bensulfuron methyl.

B.7.4 Use pattern

It is intended to support the use of the herbicide bensulfuron methyl on rice in countries listed in

It is intended to support the use of the herbicide bensulfuron methyl on rice in countries listed in Table B.7.17, both on flooded and saturated soil conditions, both in pre-sowing and in post-emergence application. The intended use patterns are listed below in Table B.7.17.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 17: Good Agricultural Practice (GAP) for the 60WG product containing bensulfuron methyl

Crop and/or situation (a)	Member State or Country	Product name(s)	F/G or I (b)	Pests or Group of pests controlled I	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	# min max (k)	kg as/ha min max	water L/ha min - max	kg as/ha min-max	(l)	(m)
Rice	Italy Spain France (South) Greece Portugal Hungary	Londax® Sectar® Testar®	F	Broadleaved weeds and Cyperaceae	WG	600 g/kg	Flooded Rice Broadcast terrestrial	Pre-sowing application 5-2 days before sowing. Post-emergence Application to be made between 20 to 45 days after sowing. (Depending on locale, this may be between BBCH12 two leaves and BBCH 29 maximum tillers)	1	N/A	200-400	0.048-0.060	Normal Maturity (BBCH 89)	Flooded: (5-10 cm) water Do not apply within 60 days of harvest.
Rice	Italy Spain France (South) Greece Portugal Hungary	Londax® Sectar® Testar®	F	Broadleaved weeds and Cyperaceae	WG	600 g/kg	Saturated soil with 0-1 cm of water Broadcast terrestrial	Post-emergence Application to be made between 20 to 50 days after sowing. (Depending on locale, this may be between BBCH 12 two leaves and BBCH 29 maximum tillers)	1	N/A	200-400	0.048-0.060	Normal Maturity (BBCH 89)	Soil will be flooded with 5-10 cm of water from 2 to 5 days after the treatment and water will be kept in rice paddy for at least 15 days. Do not apply within 60 days of harvest.

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I) Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(c) e.g., biting and suckling insects, soil born insects, foliar fungi, weeds

(d) e.g., wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes - GIFAP Technical Monograph No. 2, 1989

(f) All abbreviations must be explained

(g) Method, e.g., high-volume spraying, low-volume spraying, spreading, dusting, drench

(h) Kind, e.g., overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.

(i) g/kg or g/L

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997)

(k) The minimum and maximum number of applications possible under practical conditions of use must be provided

(l) PHI - minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7.5 Identification of critical GAPs

The two GAPs consider flooded and saturated land rice cultivation. The treatments and the soil conditions are similar, moreover results from the supervised field trials in B.7.6.2 show residues always below the LOQ values. The intended uses form the critical GAPs for application.

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)**B.7.6.1 Stability of residues during storage of samples: rice grain and rice straw freezer storage stability studies**

- 4) *Freezer storage stability of DPX-F5384 in rice straw samples. Dupont unpublished report No. AMR 3056-94, January 15, 1997; Devine, T.J., 1997. Dates of experimental work: August 3, 1994 to February 2, 1996.*

Cross reference IIA, 6.0./01

Guidelines:

U.S. EPA Residue Chemistry Test Guidelines OPPTS 860.1380, Storage Stability Data.

Deviations: None

GLP:

Yes, EPA Good Laboratory Practice Standards (40 CFR Part 160).

The study is acceptable.

Materials and Methods:

The test conditions of the storage stability study on bensulfuron methyl were as follows:

Test substance:	DPX-F5384 technical; batch F5384-83; purity 99.5%
Fortification level:	0.20 mg/kg
Plant matrices:	rice straw
Storage temperature:	≤ -20 °C
Storage intervals:	0, 1, 6, 12, and 18 months

Analytical Method: reverse phase HPLC using a column- and eluent-switching system with UV detection at 254 nm; According to the method in Dupont Report AMR 2722-93 “Analytical Method for the Determination of Bensulfuron Methyl Residues in Rice Grain and Rice straw by HPLC using a column and Eluent Switching System” (*cross reference IIA, 4.2.1/09*).

The stability of bensulfuron methyl residues in rice straw was studied by fortifying untreated rice straw specimens with bensulfuron methyl at a concentration of 0.20 mg/kg. Analysis of the 0-day unfortified control sample yielded <0.05 ppm DPX-F5384, indicating that no significant background levels of DPX-F5384 were present in the test system before the beginning of the study. Two grams of unfortified rice straw was weighted into polypropylene bags. To prepare the aged fortified samples, 1 sample were fortified with 400mL of the stock solution of DPX-F5384 in acetonitrile (1µg/mL). This corresponded to a fortification level of 0.20 mg/kg DPX-F5384. Specimens were analyzed on Day 0 (prior to freezing) and after 1, 6, 12, and 18 months of frozen storage at approximately -20°C.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Two fortified and two control samples were removed from frozen storage at each specified time interval. Bensulfuron methyl was analyzed by reverse phase HPLC using column and eluent switching with UV detection at 254 nm. The LOQ of this method is 0.05 ppm for rice straw. According to the EU Guidelines, residue levels in the fortified material should be at least 10 times higher than the LOQ of the analysis method, so that any degradation can be accurately demonstrated. Notwithstanding this rule, also the lower fortification level provided good stability results.

Findings:

Bensulfuron methyl was stable in rice straw specimens for at least 18 months when stored in a freezer at approximately -20°C prior to analysis. Normalised bensulfuron methyl residue recoveries in rice straw ranged from 74-89%. Detailed results are presented in Table B.7. 18.

Table B.7. 18: Storage stability of bensulfuron methyl in rice straw

		% Recovery				
Crop commodity	Approximate storage interval	Freezer stored fortifications	Fresh fortifications	Average recovery		Normalised recovery ^a
	(months)			Stored	Fresh	
Rice straw	0	---	73, 76, 74	---	74 ± 2	74 ^b
	1	78, 79	101	78	101	77
	6	86, 83	84	84	105	80
	12	80, 79	89	79	89	89
	18	81, 84	96	82	96	85

^aNormalised Recovery = (average stored % recovery/fresh fortification % recovery) X 100

^bNormalised Recovery = average of all fresh fortification recoveries

Conclusion:

Residues of bensulfuron methyl were stable for at least 18 months in rice straw following frozen storage at approximately -20°C.

- 5) *Storage stability of bensulfuron methyl in rice grain; Data summary in support of the registration of Londax[®] 60DF on rice crops in southern Europe. Nathan III, E.C., 2002. Dupont unpublished report No. 8797, Dates of experimental work: March 1, 1984 to February 22, 1988.*

Cross reference IIA, 6.0./02

Guidelines:

No

GLP:

The study was not done in compliance with GLP since these requirements were not in place at the time.

The study is acceptable.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Materials and Methods:

The test conditions of the storage stability study on bensulfuron methyl were as follows:

Test substance:	DPX-F5384 technical, purity and batch not specified.
Fortification level:	0.10 mg/kg
Plant matrices:	rice grain
Storage temperature:	≤ -24 °C
Storage intervals:	0, 32, 97, 200, 371, 568, 737, 1115 and 1301 days

Analytical Method: normal phase HPLC using photoconductivity detection. (According to the method in Dupont Report AMR 132-83; *Cross reference IIA, 4.2.1/05*).

Whole grain rice was ground to a coarse powder and 25-g samples were pre-weighted into glass bottles with inert plastic caps. Two samples of rice were fortified at time zero with 2.5 µg of DPX-F5384 from a 2.5 µg/mL standard solution in methylene chloride. After evaporating the methylene chloride, the fortified samples were stored frozen at approximately -24°C. Specimens were analyzed on Day 0 (prior to freezing) and after 32, 97, 200, 371, 568, 737, 1115, and 1301 days (42 months) of frozen storage. For analysis intervals other than Day 0, one frozen control sample fortified with bensulfuron methyl just prior to analysis and two frozen samples fortified with bensulfuron methyl at the beginning of the study (Day 0) were analyzed.

The Limit of Quantification of the method was 0.02 ppm.

Findings:

The data on recoveries are presented in Table B.7. 19. Bensulfuron methyl was stable in rice grain specimens for at least 1301 days (42 months) when stored in a freezer at approximately -24°C prior to analysis. Normalised bensulfuron methyl residue recoveries in rice grain were 91% or greater (range 91-133%) showing sufficient method performance.

Table B.7. 19: Storage stability of bensulfuron methyl in rice grain

		% Recovery				
Crop commodity	Approximate storage interval	Freezer stored fortifications	Fresh fortifications	Average recovery		Normalised recovery ^a
	Days (months)			Stored	Fresh	
Rice grain	0 (0)	---	92, 90, 98	---	93 ± 4	93 ^b
	32 (1.1)	84, 84	90	84	90	93
	97 (3.2)	93, 90	95	91	95	96
	200 (6.6)	94, 83	97	88	97	91
	371 (12.2)	88, 87	88	87	88	99
	568 (18.7)	94, 79	77	86	77	112
	737 (24.2)	142, 93	104	117	104	112
	1115 (36.7)	87, 89	89	88	89	99
	1301 (42.8)	112, 74	70	93	70	133

^a Normalised recovery = (average stored % recovery/fresh fortification % recovery) X 100

^b Normalised recovery = average of all fresh fortification recoveries

Conclusion:

Residues of bensulfuron methyl were stable for at least 42 months in rice grain following frozen storage at approximately -24°C.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

- 6) *Magnitude of residues of bensulfuron methyl in rice (cereals) following application of Bensulfuron methyl 60WG formulation in paddy rice fields - southern Europe, season 2000. Weber, H., Bramble Jr, F.Q., 2002. Dupont unpublished report No. AMR 4139; Dates of experimental work: December 27, 2000 to April 4, 2002.*

Cross reference IIA, 6.0./03; 6.3/04

Guidelines:

EU 1607/VI/97 Rev 1 (22/7/1997), Appendix B, Residue Trials, 7029/VI/95 Rev 5 (22/7/1997), Appendix H, Storage Stability of Residues Samples, 7032/VI/95 (22/7/97).

Deviations: None

GLP:

Yes, "OECD Principles of Good Laboratory Practice (as revised in 1997)" and the GLP Consensus Document "The Application of the GLP Principles to Field Studies".

The study is acceptable.

Materials and Methods:

The test conditions of the storage stability study on bensulfuron methyl were as follows:

Test substance:	DPX-F5384 60WG; batch F5384-332;
Fortification level:	0.10 mg/kg in rice and 0.50 mg/kg in straw
Plant matrices:	rice grain and straw
Storage temperature:	≤ -20 °C
Storage intervals:	0, 12, and 15 months

Analytical Method: reverse phase HPLC using a column-switching system with UV detection at 254 nm (according to the method in Report No. Dupont-4510, *cross reference IIA, 4.2./01*).

This fortification study was performed concurrently with a field study in paddy rice fields (*cross reference IIA, 6.3/04*).

The stability of bensulfuron methyl residues in rice grain and rice straw was studied by fortifying untreated rice grain and straw specimens with bensulfuron methyl at a concentration of 0.10 and 0.50 mg/kg, respectively. Specimens were analysed on Day 0 (prior to freezing) and after 12 and 15 months of frozen storage at approximately -20°C. Bensulfuron methyl was analysed by reverse phase HPLC using column switching with UV detection at 245 nm. The Limit of Quantification established for the analytical method was 0.010 mg/kg for rice grain and 0.050 mg/kg for rice straw.

Findings:

All normalised recoveries of bensulfuron methyl residues in rice grain and straw ranged from 75-107%. These results demonstrate sufficient method performance and bensulfuron methyl stability in specimens stored in a freezer for at least 15 months at approximately -20°C prior to analysis. Detailed results are presented in Table B.7. 20.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 20: Storage stability of bensulfuron methyl in rice grain and straw

		% Recovery				
Crop commodity	Approximate storage interval	Freezer stored fortifications	Fresh fortifications	Average recovery		Normalised recovery ^a
	(months)			Stored	Fresh	
Rice grain	0	---	92, 98	---	95	95 ^b
	12	66, 65	85, 79 ^c	66	82	80
	15	67, 66	68, 77	67	73	92
Rice straw	0	---	107, 106		107	107 ^b
	12	77, 78	64, 109 ^c	78	87	90
	15	81, 78	106, 107	80	107	75

^a Normalised recovery = (average stored % recovery/average fresh fortification % recovery) X 100^b Normalised recovery = average of all fresh fortification recoveries^c Fresh fortification recoveries in grain or straw were from the previous day's method validation analysis**Conclusion:**

Residues of bensulfuron methyl were stable for at least 15 months in rice grain and straw following frozen storage at approximately -20°C.

Overall conclusion on storage stability

Bensulfuron Methyl is stable in rice grain up to 42 months at -24°C and in rice straw up to 18 months at -20°C.

B.7.6.2 Supervised residue trials

The magnitude of bensulfuron methyl residues was monitored in a total of 14 field residue trials, representing pre-harvest uses on rice: 10 trials on flooded rice and 4 trials on dry land rice. These trials were conducted over five growing seasons, 1991, 1995, 1996, 2000, and 2002 in locations representative of major rice production areas in the southern European region, using representative Bensulfuron methyl WG formulations. The study conducted in Greece in 1991 (*cross reference IIA, 6.3/03*) was not performed in compliance with the proposed GAP and no adequate information is given on the field phase of the trial. For these reasons the study was considered not acceptable in support of the registration of bensulfuron methyl.

Locations of the trial sites are presented in Figure B.7. 6 and summarised in Table B.7. 21.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Figure B.7. 6: Map: Bensulfuron methyl European rice test sites



Table B.7. 21: Summary of the locations of trials to determine residues of bensulfuron methyl in rice

No.	Location	No.	Location
<u>Magnitude of Residue Trials</u>		<u>Magnitude of Residue Trials</u>	
1	Greece: Kalohori, Thessaloniki	8	Italy: S. Martino Siccomario, Pavia
2	Italy: Ceretto Lomellina, Pavia	9	Italy: Zibido S. Giacomo, Milano
3	Italy: Villarboit, Vercelli	10	Spain: Villafranco, Sevilla
4	Italy: Rosate, Milano	11	Italy: S. Martino, Siccomario, Pavia
5	Italy: Ceretto Lomellina, Pavia	12	Italy: Mulazzano, Lombardia
6	Italy: Villarboit, Vercelli	13	Spain: La Puebla de Río, Sevilla
7	Italy: Rosate, Milano	14	Spain: Villafranco del Guadalquivir, Sevilla

- 7) *Field dissipation of bensulfuron methyl and metsulfuron methyl from a representative rice field following treatment of three consecutive years with Sigma 60 DF herbicide in Italy. Season 1994-1996. Bonacini, F., Zenide, D., Gasser, F., Leka, A. Unpublished DuPont report No. AMR 4162-96 (I-94-003-DIS) December 1, 1998.*

Cross reference IIA, 6.3./01

and

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

- 8) *Field dissipation of bensulfuron methyl and metsulfuron methyl from a representative rice field following treatment of three consecutive years with Sigma 60 DF herbicide in Italy, Seasons 1994-1996, and magnitude of residues in rice grains. Bonacini, F., Zenide, D., Gasser, F., Leka, A., 1999. Unpublished DuPont report No. AMR 4162-96 (I-94-003-DIS) December 1, 1998 Amendment No.1.*

Cross reference IIA, 6.3./02

Guidelines:

OECD/GD(92)37, Environmental Monograph No. 50, Paris 1992 Protocol.

GLP:

The study was conducted according to the OECD Principles of Good Laboratory Practice published in the OECD/GD(92)32, Environmental Monograph No. 45 and OECD/GD(92)37, Environmental Monograph 50.

The study is acceptable.

Material and methods:

Test Substance: DPX-JM206 60WG (Sigma 60DF)

Batch: IN-JM206

Content of a.i.: 58% bensulfuron methyl, 2% metsulfuron methyl

Crop: Rice (flooded)

Field information:

Three test locations during both the 1995 and 1996 field seasons in the southern European region.

Italy: Ceretto-Lomellina, Pavia

Italy: Villarboit, Vercelli

Italy: Rosate, Milano

Test substance: DPX-JM206 60WG formulation

(contains 58% bensulfuron methyl a.s. + 2% metsulfuron methyl a.s., 60% total a.s., w/w)

Application timing: One application was made at each of the three test sites per year (1995 and 1996) at the end of tillering rice growth stage (BBCH 29)

Type of application: overall spray application on flooded soil

Rate of Application: 54 to 61 g bensulfuron methyl a.s./ha

Number of applications/season: 1

Spray volume: 244 to 365 L/ha

Vehicle/solvent: Water only

Additive: None

Sampling:

Sampling interval: 91 to 112 days after application

Commodity portion sampled: Mature rice grain

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Sample storage:

Field storage/shipping temperature: Frozen <-20°C

Analytical facility storage temperature: Approximately -18°C

Storage stability:

Bensulfuron methyl residues are stable under frozen storage conditions in rice grain for at least 42 months following storage at approximately -24°C. Samples were analysed 15 months after being harvested.

Analysis method: The rice grains samples were ground in a grain grinder. To minimize the risk of contamination the control samples were prepared first, followed by the treated ones. The samples were returned to the freezer until the time of the extraction procedure.

After extraction from the rice grain samples with methylene chloride and different partition procedures and clean up on C-18 IST-ISOLUTE™ SPE cartridge, the determination of bensulfuron methyl residues was carried out by liquid chromatography with photoconductivity detector (HPLC/PCD).

Recovery data: Average recovery data for fortifications run concurrently with the treated specimens are given in Table B.7. 22

Table B.7. 22: Recovery data for bensulfuron methyl in rice grain

Crop fraction	Fortifications (mg/kg)	Number of samples	Percent recovery (mean ± S.D.)
Grain	0.020	3	73 ± 5
Grain	0.10	3	86 ± 6
	Average	6	79 ± 9

Method limits: Limit of quantification is 0.020 mg/kg based on a 25-gram sample. The limit of detection is estimated to be 0.007 mg/kg based on examination of chromatograms from the study.

Findings:

Residue results: Bensulfuron methyl residues were expressed as mg/kg of rice grain. No quantifiable residues (<0.020 mg/kg) of bensulfuron methyl were found at crop maturity (91-112-day PHI/DALA) in flooded rice grain specimens treated with 54-61 g bensulfuron methyl a.s./ha after harvest from the three field locations in this study (total of 6 field trials over the 1995 and 1996 field seasons). Recovery and control specimens analysed concurrently with treated specimens indicated that the method generated acceptable data. A residue summary table is presented in Table B.7. 23.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 23: Summary of average bensulfuron methyl residues in rice grain (mg/kg)-southern Europe, seasons 1995 and 1996

Report N°, Country, year	Crop / Variety	Formulation tipe	Method of treatment	Application rate					Portion analyzed	Residues (mg/kg)	PHI (days after application)
				No.	kg ^b as/ha	Water (l/ha)	kg as/hl	Growth stage			
I-94-03-DIS Italy , Cereto-Lomellina, Pavia, 1995	Rice / Drago	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.060	244	0.025	BBCH 29	Grain	<0.02	110
I-94-03-DIS Italy , Villarboit, Vercelli, 1995	Rice / Panda	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.057	354	0.016	BBCH 29	Grain	<0.02	108
I-94-03-DIS Italy , Rosate, Milano, 1995	Rice / Panda	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.061	365	0.017	BBCH 29	Grain	<0.02	99
I-94-03-DIS Italy , Cereto-Lomellina, Pavia, 1996	Rice / Drago	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.057	256	0.022	BBCH 29	Grain	<0.02	91
I-94-03-DIS Italy , Villarboit, Vercelli, 1996	Rice / Panda	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.059	354	0.017	BBCH 29	Grain	<0.02	100
I-94-03-DIS Italy , Rosate, Milano, 1995	Rice / Panda	DPX-JM206 60WG ^d	Overall spray in flooded soil	1	0.054	340	0.016	BBCH 29	Grain	<0.02	112

^a Duplicate field specimens were taken from each site. Specimens were analysed separately. Averaged results are reported.

^b kg a.s./ha of bensulfuron methyl. DPX-JM206 60WG contains 58% bensulfuron methyl and 2% metsulfuron methyl (60% total a.s.)

^d DPX-JM206 60WG= Sigma 60DF

Conclusion

No quantifiable residues of bensulfuron methyl were found in rice grain at harvest following treatment with 56-64 g a.s./ha of bensulfuron methyl applied as DPX-JM206 60WG.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

- 9) *Determination of residues of bensulfuron methyl (DPX-F5384) in rice grain by HPLC-PCD following treatment with Londax® 60WG (Greece 1991). Unpublished report No. BE-A-11-92-09-BG, June 3, 1992. Cicotti, M., 1992.*

Cross reference IIA, 6.3./03

Guidelines:

No

GLP:

The study was conducted in compliance with the OECD C(81) 30 final, May 12, 1981, Good Laboratory Practices regulations.

The study is not acceptable since not performed in compliance with the proposed GAP and no adequate information is given on field phase of the trial.

Material and methods

Test Substance: DPX-F5384 – bensulfuron methyl (Londax 60 WG)

Crop: Rice (flooded).

Field information:

One supervised residue trial was performed in Greece (southern European region) in 1991. The test information are summarized below.

Crop / cultivar	Rice (flooded), cultivar Ispaniki A
Test substance:	Bensulfuron methyl 60WG formulation
Application timing:	14-June-1991, early post application at 2-3 leaf growth stage (BBCH13)
Type of application:	spraying in flooded paddy, after sowing
Number of plot:	1 control and 2 treated plots
Rates of application:	One plot at 60 and one plot at 120 g bensulfuron methyl a.s./ha
Number of applications:	1/treated plot
Spray Volume:	350 L/ha
Vehicle/solvent:	Water only
Additive	None

Sampling:

Date of sampling:	5 Nov 1991
Sampling interval:	144 days after application
Commodity portion sampled:	Mature rice grain
Sample size	1 kg
Field storage/shipping temperature:	Approximately –18°C

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Storage stability:

The samples were stored at –20 °C and defrosted before analysis.

Bensulfuron methyl residues are stable under frozen storage conditions in rice grain for at least 42 months following storage at approximately –24°C. Specimens were analysed 5 months after being harvested.

Analysis method:

Analysis method:	normal phase HPLC-PCD method
Analyte:	Bensulfuron methyl
Extraction:	Methylene chloride
Clean-up:	C-18 IST-ISOLUTE™ SPE cartridge
Chromatography:	Normal phase HPLC
Detection:	Photoconductivity detection (PCD)

Recovery data: Average recovery data for a fortification run concurrently with treated specimens is given in Table B.7. 24.

Table B.7. 24: Recovery data for bensulfuron methyl in rice grain

Crop fraction	Fortification (mg/kg)	Number of samples	Percent recovery
Grain	0.020	1	103

Method limits: The limit of quantification was 0.020 mg/kg based on a 25-g sample.

Findings:

Residue results: No quantifiable residues (<0.020 mg/kg) of bensulfuron methyl were found at crop maturity (144-day PHI/DALA) in flooded rice grain specimens treated with either 60 or 120 g a.s./ha (1X and 2X the GAP rate) after harvest from the field location in this study. Recovery and control specimens analysed concurrently with treated specimens indicated that the method generated acceptable data. A residue summary table is presented in Table B.7. 25.

Table B.7. 25: Summary of average bensulfuron methyl residues in rice grain (mg/kg)-southern Europe, season 1991

Report N°, Country, year	Crop Variety	Formulation type	Method of treatment	Application rate					Portion analyzed	Residues (mg/kg)	PHI (days)
				No.	kg as/ha	Water (l/ha)	kg as/hl	Growth stage			
BE-A-11-92-09-BG Greece, Kalohori, Thessaloniki, 1991	Rice / Ispaniki A	Bensulfuron methyl 60WG	spraying in flooded paddy	1	0.060	350	0.017	Early post 2-3 leaves BBCH 13	Grain	ND	144
				1	0.120	350	0.034		Grain	ND	144

Conclusion:

No quantifiable residues of bensulfuron methyl were found in rice grain at harvest following treatment with 60 or 120 g a.s./ha of Bensulfuron methyl 60WG (1X and 2X the GAP rate, respectively).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

10) *Magnitude of residues of bensulfuron methyl in rice (cereals) following application of Bensulfuron methyl 60WG formulation in paddy rice fields - southern Europe, season 2000. Unpublished report No. DuPont-4139, June 14, 2002, Weber, H., Bramble Jr, F.Q., 2002.*

Cross reference IIA, 6.3./04; 6.0/03

Guidelines:

EU Commission Working Document 1607/VI/97 Rev 1 (22/7/1997), Appendix B, Residue Trials, 7029/VI/95 Rev 5 (22/7/1997); Appendix H, Storage Stability of Residues Samples, 7032/VI/95 (22/7/97)

Deviations: None

GLP:

The study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in the ENV/MC/CHEM(98)17 Unclassified Consensus document, and the GLP Consensus Document "The Application of the GLP Principles to Field Studies" which are essentially consistent with the US EPA GLP Standard (40 CFR Part 160).

The study is acceptable

Material and methods

Test Substance: DPX-F5384 60WG (Commercial name: Londax)

Batch: F5384-332

Crop: flooded rice

Field information:

Four supervised trials were carried out during the 2000 field season in the southern European region, comparing one treated plot of rice to one control plot. The specifics of the field trials are summarized below.

Test location	Test No. 1: S. Martino Siccomario, Pavia (Italy) Test No. 2: Zibido S. Giacomo, Milano (Italy) Test No. 3: Catarroja, Valencia (Spain) Test No. 4: Villafranco, Sevilla (Spain)
Test substance	Bensulfuron methyl 60WG formulation; commercial name: Londax
Application timing:	26-June-2000 to 05-July-2000, BBCH 32
Type of application:	Ground directed application
Number of applications:	1
Rate of application:	58-61 g bensulfuron methyl a.s. /ha
Spray volume:	388 - 412 L/ha
Vehicle/solvent:	Water only
Additive:	None

The test No. 3 in Valencia, Spain was invalidated because the harvest was made 38 days after the application, while the minimum interval between treatment and harvest (PHI) specified in the GAP is 60 days. For this reason the residue results from the Catarroja test site are not included in the summarised results.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Sampling:

The rice crop at each test site was harvested at normal maturity. The time to maturity after treatment varied in each test with different rice varieties and environmental conditions in a range of 69-95 days. At least 1 kg of rice grain specimen and 0.5 kg of rice straw specimen were collected separately from the control and treated plot at each site from the harvested crop.

Control and treated grain and straw samples – identified by unique codes, were stored under frozen conditions (-18 °C) after collection from the field and during the shipment.

Storage stability:

The samples were stored at or less than -18 °C for no longer than 127 days (4.2 months) between sampling and extraction. The results from the storage stability test conducted concurrently with the analyses in this study supported the stability of Bensulfuron methyl stored frozen in rice grain and straw for up to 15 months.

Analysis:

Bensulfuron methyl residues were determined using column switching high performance liquid chromatography (HPLC) with ultraviolet detection.

The Limit of Quantification (LOQ) established for the analytical method was 0.01 mg/kg for rice grain and 0.05 mg/kg for rice straw.

Analyte:	Bensulfuron methyl
Extraction:	50-mM ammonium carbonate buffer
Clean-up:	Envi TM -Carb SPE cartridge
Chromatography:	Column switching reversed phase HPLC
Detection:	Ultraviolet detector

Recovery data: Average recovery data for fortifications run concurrently with the treated specimens are given in Table B.7. 26.

Table B.7. 26: Recovery data for bensulfuron methyl in rice grain and straw

Crop fraction	Fortifications (mg/kg)	Number of samples	Percent recovery (mean ± S.D.)
Rice grain	0.010	3	99 ± 8
	0.10	3	94 ± 4
	Average	6	96 ± 6
Rice straw	0.050	3	103 ± 3
	0.50	3	103 ± 6
	Average	6	103 ± 4

Method limits: The limit of quantification was 0.010 mg/kg in rice grain and 0.050 mg/kg in rice straw.

Findings:

Residue results: Bensulfuron methyl residues were expressed as mg bensulfuron methyl/kg of rice grain or straw. No quantifiable (<0.010 mg/kg) residues of bensulfuron methyl were found at crop maturity (69-95-day PHI/DALA) in flooded rice grain specimens treated with 58-61 g a.s./ha after harvest from the three field locations in this study. For these supervised field trials, bensulfuron methyl was applied at growth stage 32 (BBCH 32) slightly later than growth stage 29 specified by GAP. Since all field trials showed no quantifiable residues in rice grain and represent

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

application timing more conservative (later) than required by GAP, there is no reasonable expectation of bensulfuron methyl residue occurring in rice grain at normal harvest when application is made with timing specified by GAP. Residues of bensulfuron methyl in rice straw were also below the limit of quantification of the analytical method (<0.050 mg/kg). Recovery and control specimens analysed concurrently with treated specimens indicated that the method generated acceptable data. A residue summary table is presented in Table B.7. 27.

Table B.7. 27: Summary of average bensulfuron methyl residues in rice grain (mg/kg)-southern Europe, season 2000

Report N°, Country, year	Crop / Variety	Formulation type	Method of treatment	Application rate					Portion analyzed	Residues (mg/kg)	PHI (days)
				No.	kg as/ha	Water (l/ha)	kg as/hl	Growth stage			
DuPont-4139 Italy , S. Martino Siccomario, Pavia, 2000	Rice / Loto	Bensulfuron methyl 60WG	Ground directed application	1	0.060	403	0.015	BBCH 32	Grain Straw	< 0.01 < 0.05	77
DuPont-4139 Italy , Zibido S. Giocomo, Milano, 2000	Rice / Loto	Bensulfuron methyl 60WG	Ground directed application	1	0.058	388	0.015	BBCH 32	Grain Straw	< 0.01 < 0.05	69
DuPont-4139 Spain , Villafranco, Sevilla, 2000	Rice / Thaibonet	Bensulfuron methyl 60WG	Ground directed application	1	0.061	412	0.015	BBCH 32	Grain straw	< 0.01 < 0.05	95

Conclusion:

No quantifiable residues of bensulfuron methyl were found in rice grain and straw at harvest following treatment with 58-61 g a.s./ha of Bensulfuron methyl 60WG.

11) *Magnitude of residues of bensulfuron methyl in rice (cereals) following application of Bensulfuron methyl (DPX-F5384) 60WG formulation in wetland rice fields - southern Europe, season 2002. Unpublished report No.DuPont-9208, April 11, 2003, Weber, H., Hornshuh, M.J., 2003.*

Cross reference IIA, 6.3./05

Guidelines:

EU Commission Working Document 1607/VI/97 Rev 2 (1999), Appendix B, Residue Trials, 7029/VI/95 Rev 5 (1997).

Deviations: None

GLP:

The study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in the ENV/MC/CHEM(98)17 Unclassified Consensus document, and the GLP Consensus Document "The Application of the GLP Principles to Field Studies" as revised in 1999 ENV/JM/MONO(99)22, which are essentially consistent with the US EPA GLP Standard (40 CFR Part 160).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

The study is acceptable

Material and methods

Test Substance: DPX-F5384 60WG (Bensulfuron methyl 60WG formulation)

Batch: DPX-F5384-258

Crop: Rice (saturated soil)

Field information:

Four test locations during the 2002 field season in the southern European region, comparing one treated plot of rice to one control plot. The specifics of the field trials are summarized below.

Trial sites:

Italy: S. Martino Siccomario, Pavia

Italy: Mulazzano, Lodi

Spain: La Puebla del Río, Sevilla

Spain: Villafranco del Guadalquivir, Sevilla

Application:

Application timing: 25-July-2002 to 02-August-2002, at the BBCH
32 growth stage

Type of application: Ground directed application

Rate of application: 58-61 g bensulfuron methyl a.s. /ha

Number of applications: 1

Spray volume: 250 - 408 L/ha

Vehicle/solvent: Water only

Additive: None

Sampling:

Sampling interval: 67-80 days after application

Commodity portion sampled: Mature rice grain and straw

Sample storage:

Field storage/shipping temperature: <-18°C/<-18°C

Analytical facility storage temperature:: Approximately -20°C

Storage stability:

Bensulfuron methyl residues are stable under frozen storage conditions in rice grain for at least 42 months following storage at approximately -24°C. Bensulfuron methyl was stable in rice straw for at least 18 months following storage at approximately -20°C. Samples of rice grain and straw were stored no longer than 4 months before analysis.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Analysis method:

Analyte: Bensulfuron methyl
 Extraction: 50-mM ammonium carbonate buffer
 Clean-up: Envi™-Carb SPE cartridge
 Chromatography: Reversed phase HPLC
 Detection: HPLC/MS/MS

Recovery data: Average recovery data for fortifications run concurrently with treated samples are given in Table B.7. 28.

Table B.7. 28: Recovery data for bensulfuron methyl in rice grain and straw

Crop fraction	Fortifications (mg/kg)	Number of samples	Percent recovery (mean ± S.D.)
Grain	0.010	2	106
	0.10	2	96
Straw	0.050	2	100
	0.50	2	104
	Overall	8	102 ± 6

Method limits: Limit of quantification is 0.010 mg/kg in rice grain and 0.050 mg/kg in rice straw.

Findings:

Residue results: No quantifiable residues (<0.01 mg/kg) of bensulfuron methyl were found at crop maturity (67-80-day PHI/DALA) in rice grain specimens grown under wetland field conditions following treatment with 58-61 g a.s./ha from the four field locations in this study. For these supervised field trials, bensulfuron methyl was applied at growth stage 32 (BBCH 32) slightly later than growth stage 29 specified by GAP. Since all field trials showed no quantifiable residues in rice grain and represent application timing more conservative (later) than required by GAP, there is no reasonable expectation of bensulfuron methyl residue occurring in rice grain at normal harvest when application is made with timing specified by GAP. Residues of bensulfuron methyl in rice straw varied from 0.10 mg/kg to below the limit of quantification (<0.05 mg/kg) of the analytical method.

Recovery and control specimens analysed concurrently with treated specimens indicated that the method generated acceptable data. A residue summary table is presented in Table B.7. 29.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 29: Summary of average bensulfuron methyl residues in rice grain and straw (mg/kg) southern Europe, season 2002

Report N°, Country, year	Crop / Variety	Formulation tipe	Method of treatment	Application rate					Portion analyzed	Residues (mg/kg)	PHI (days)
				No.	kg as/ha	Water (l/ha)	kg as/ha	Growth stage			
DuPont-9208 Italy, Martino Siccomario, Pavia, 2002	Rice / Loto	Bensulfuron methyl 60WG	Ground directed application	1	0.061	408	0.015	BBCH 32	Grain Straw	<0.01 <0.05	76
DuPont-9208 Italy, Maluzzano, Lodi, 2002	Rice / Baldo	Bensulfuron methyl 60WG	Ground directed application	1	0.058	384	0.015	BBCH 32	Grain Straw	<0.01 <0.05	67
DuPont-9208 Spain, La Puebla del Rio, Sevilla, 2002	Rice / Puntual	Bensulfuron methyl 60WG	Overall application	1	0.060	250	0.024	BBCH 32	Grain Straw	<0.01 0.10	78
DuPont-9208 Spain, Villafranco del Guadalquivir, Sevilla, 2002	Rice / Thaigonet	Bensulfuron methyl 60WG	Overall application	1	0.060	351	0.017	BBCH 32	Grain Straw	<0.01 <0.05	80

Conclusion:

No quantifiable residues of bensulfuron methyl were found in rice grain at harvest following treatment with 58-62 g a.s./ha of Bensulfuron methyl 60WG. Residues of bensulfuron methyl in rice straw ranged from 0.10 mg/kg to not quantifiable (<0.05 mg/kg).

B.7.6.3 Overall conclusions on residues resulting from supervised trials

The magnitude of bensulfuron methyl residues was monitored in a total of 14 field residue trials, representing pre-harvest uses on rice: 10 trials on flooded rice and 4 trials on saturated land rice. These trials were conducted over five growing seasons, 1991, 1995, 1996, 2000, and 2002 in locations representative of major rice production areas in the southern European region, using representative Bensulfuron methyl WG formulations.

Eight residue trials were conducted using Bensulfuron methyl 60WG; six residue trials were conducted using DPX-JM206 60WG (contained 58% a.s. bensulfuron methyl + 2% a.s. metsulfuron methyl).

When bensulfuron methyl is applied with timing specified by GAP (BBCH 29), there are no quantifiable residues in rice grain. The likelihood of no residues in rice grain at normal harvest is further strengthened by evidence of no quantifiable residue in rice grain when applications are made at timing slightly beyond the BBCH 29 growth stage (BBCH 32). Residues in rice grain were consistently below the LOQ of the analytical methods used for residue analysis [0.02 or 0.01 mg/kg] for all residue field trials. All residues were determined using validated methods, which produced good recovery data for fortified control specimens run concurrently with treated specimens.

Since no quantifiable residue of bensulfuron methyl was observed in rice grain under worst case GAP conditions or conditions more restrictive than required by GAP (field applications at the BBCH 32 rather than BBCH 29 stage of growth), no residue decline trials were conducted.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

The GAP for use of bensulfuron methyl on flooded or saturated land rice specifies application of up to 60 g a.s./ha as late as growth stage 29 (BBCH 29, end of tillering). Application timing must not produce a PHI shorter than 60 days. Supervised residue field trials supporting re-registration of bensulfuron methyl were conducted in general compliance with GAP. However, some bensulfuron methyl applications for supervised field trials conducted during 2000 and 2002 were applied at growth stage 32 (BBCH 32, panicle formation: panicle 1-2 mm in length). As this timing is slightly beyond the GAP recommendation of growth stage 29, these trial results represent a more conservative position. All other parameters, for these trial installations, complied with GAP.

Since the study conducted in Greece in 1991 (*cross reference IIA, 6.3/03*) was evaluated not acceptable, the residue data from it are not used for the calculation of MRL. The residue data from grain field trials performed in compliance with the GAP are sufficient for the calculation of MRL and are listed in Table B.7. 30.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 30: Summary of bensulfuron methyl accepted residues (mg/kg) in rice: growing seasons 1995, 1996, 2000 and 2002

Country, location, year	Application					Application timing	Residue	Reference
	Test Material	No	kg a.s./ ha	kg a.s./ hL	Crop portion analysed	(days after last application)	(mg/kg)	
Flooded soil conditions								
Italy, Ceretto Lomellina, 1995	DPX-JM206 60WG ^a	1	0.060	0.027	Grain	110	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, Villarboit, 1995	DPX-JM206 60WG ^a	1	0.057	0.016	Grain	108	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, Rosate, 1995	DPX-JM206 60WG ^a	1	0.058	0.016	Grain	99	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, Ceretto Lomellina, 1996	DPX-JM206 60WG ^a	1	0.057	0.022	Grain	91	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, Villarboit, 1996	DPX-JM206 60WG ^a	1	0.059	0.017	Grain	100	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, Rosate, 1996	DPX-JM206 60WG ^a	1	0.054	0.016	Grain	112	<0.02	AMR 4162-96 <i>IIA, 6.3/01-02</i>
Italy, S. Martino Siccomario, 2000	Bensulfuron methyl 60WG	1	0.060	0.015	Grain Straw	77	<0.01 <0.05	DuPont-4139 <i>IIA, 6.3/04</i>
Italy, Zibido S. Giacomo, 2000	Bensulfuron methyl 60WG	1	0.058	0.015	Grain Straw	69	<0.01 <0.05	DuPont-4139 <i>IIA, 6.3/04</i>
Spain, Villafranco, 2000	Bensulfuron methyl 60WG	1	0.061	0.015	Grain Straw	95	<0.01 <0.05	DuPont-4139 <i>IIA, 6.3/04</i>
Saturated soil conditions								
Italy, S. Martino Siccomario, 2002, saturated land	Bensulfuron methyl 60WG	1	0.061	0.015	Grain Straw	76	<0.01 <0.05	DuPont-9208 <i>IIA, 6.3/05</i>
Italy, Mulazzano, 2002 saturated land	Bensulfuron methyl 60WG	1	0.058	0.015	Grain Straw	67	<0.01 <0.05	DuPont-9208 <i>IIA, 6.3/05</i>
Spain, La Puebla de Río, 2002 saturated land	Bensulfuron methyl 60WG	1	0.060	0.024	Grain Straw	78	<0.01 0.10	DuPont-9208 <i>IIA, 6.3/05</i>
Spain, Villafranco, 2002 saturated land	Bensulfuron methyl 60WG	1	0.060	0.017	Grain Straw	80	<0.01 <0.05	DuPont-9208 <i>IIA, 6.3/05</i>

^a DPX-JM206 60WG contains 58% bensulfuron methyl and 2% metsulfuron methyl (60% total a.s.)

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

According to the European guidelines in Appendix E doc. 7035/VI/95 rev.5 (22/7/97) processing studies are not necessary “if no significant (>0.1 mg/kg) or no analytically quantifiable residues occur in the plant product being processed”. No quantifiable bensulfuron methyl residues were found in any mature rice grain specimen at the time of harvest. Therefore, no processing study is necessary.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

According to the European guidelines in Appendix G doc. 7031/VI/95 rev.4 (22/7/96) livestock feeding studies are required when significant residues (≥ 0.1 mg/kg of the total diet received) occur in crops fed to animals. In addition,

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

livestock feeding studies are required when metabolism studies indicate that significant residues (>0.1 mg/kg) may occur in products of animal origin as a result of animals consuming feeding stuffs or fodder crops containing residues. In the case of bensulfuron methyl, livestock feeding studies are not required since anticipated residues in the diet are less than 0.1 mg/kg total diet (Table B.7. 5). There are no quantifiable residues of the active substance in mature rice grain at harvest as shown in the plant metabolism studies and crop residue studies. Feeding studies are not necessary to assess human exposure to bensulfuron methyl from animal food sources.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6; Annex III 8.5)

Bensulfuron methyl is degraded in soil relatively quickly (mean soil DT_{50} in field trials ranges from 4 to 14 days – see chapter B.8.1.3.1), therefore, it is very unlikely that significant quantities ($>10\%$ of applied active substance) will remain in the soil 100 days after application. However, a confined rotational crop study was conducted.

The total radioactive residues in the soil at the time of planting rotational crops and at harvest were ≤ 0.01 mg/kg. The majority of soil radioactivity was extracted. Unextracted radioactivity was < 0.002 mg/kg. Bensulfuron methyl was the only radioactive residue in soil samples and was present at 0.005-0.006 mg/kg. Total ^{14}C residues in food items from wheat, onions, garlic, cabbage, and beets grown in the soil treated with ^{14}C bensulfuron methyl at the rate of 67.2 g a.s./ha and aged for 120 days prior to the planting, were less than 0.01 mg/kg.

12) Crop rotation studies with ^{14}C -labeled DPX-F5384 in the greenhouse. Monson, K.D., Bullock, J.H., 1985. Unpublished study AMR-335-85.

Cross reference IIA, 6.6./01

Guidelines:

USEPA J40/9-82-021

GLP:

No GLP was required at the time of the study.

The study is acceptable as additional information.

Material and Methods:

Test Substance: ^{14}C -DPX-F5384 technical

Batch: [Pyrimidine-2- ^{14}C] HOTC number not specified; [Phenyl-U- ^{14}C] HOTC number not specified

Purity: 97% radiochemical purity

A confined rotational crop study of ^{14}C -bensulfuron methyl was conducted using Keyport silt loam soil under field and greenhouse conditions. Field pots were separately treated with either [phenyl(U)- ^{14}C]bensulfuron methyl or [pyrimidine-2- ^{14}C] bensulfuron methyl at a rate equivalent to 67.2 g a.s./ha and aged for 120 days in the field (Stine Farm, Newark, Delaware). Soil to the depth of six inches was transferred to greenhouse pots and wheat, beets, green onions, red onions, garlic, Chinese cabbage, and dwarf cabbage were planted. Crops were maintained in the greenhouse for the duration of the study.

Soil:

Soil type: Keyport silt-loam soil (from Stine Farm, Newark, Delaware)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Soil characteristics: pH 5.2, OM 7.5%, sand 12%, silt 83%, clay 5%

Growth conditions: Plants were maintained in pots and grown under greenhouse conditions (16 hr light cycle, 65-75°F, and approximately 30% relative humidity) at the DuPont Experimental Station (Wilmington, DE).

Test substance:

Phenyl(U)-¹⁴C]]bensulfuron methyl: Specific activity: 29.8 µCi/mg,
Radiochemical Purity >97%

[Pyrimidine-2-¹⁴C]bensulfuron methyl: Specific activity: 16.6 µCi/mg
Radiochemical Purity >97%

Test formulation: [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl were applied as a sand mixture containing sodium lignosulfonate as a dispersant and silica precipitate as an anti-caking ingredient.

Application rate: A single application was made directly onto the soil surface at a rate equivalent to 67.2–g a.s./hectare.

Application method: The formulated sand mixtures of [phenyl(U)-¹⁴C]bensulfuron methyl and [pyrimidine-2-¹⁴C]bensulfuron methyl were sprinkled evenly on separate test plots..

Planting intervals: After aging the treated soil for 120-days, the top six inches of soil from each radiolabeled test plot was transferred separately to a 16 inch test pot and seeds of previously described crops were planted in both the treated and control soil containers.

Sampling schedule: Soil samples to a depth of 6 inches were taken separately, prior to planting, for each radiolabel. Whole plant samples were taken at 30, 45, 60, and 105 days (final harvest) after seeding. Additional samples of wheat, green onions, and Chinese cabbage were taken at 75 days. Soil samples were taken at the final harvest.

Analysis: The amounts of total radioactive residues (TRR) in the freeze-dried and homogenised raw agricultural commodities were determined by combustion followed by liquid scintillation counting (LSC) analyses.

Soil samples were extracted using a wrist action shaker for one hour with methylene chloride/methanol/1M ammonium carbonate (100:75:25, v/v/v). The slurry was centrifuged and the extract was vacuum filtered. The procedure was repeated twice more for each radiolabel, the extracts from the same test soil were pooled, and evaporated *in vacuo* (~40°C). The concentrated extracts were acidified to pH 4 with glacial acetic acid, and partitioned three times with equal volumes of methylene chloride. The total radioactivity in the processed aqueous and organic phases was determined by LSC.

The extracted soils were refluxed three successive times, for one hour each time, with acetone/1 M ammonium carbonate (3:1, v/v). These extracts were combined, rotary evaporated, and assayed by LSC.

Aliquots of methylene chloride extracts were analysed by silica gel thin layer chromatography (TLC) using toluene/acetone/methanol/15 M ammonium hydroxide (100:100:50:2, v/v/v/v). The same samples were also analysed by C-18 TLC plates using methylene chloride/acetonitrile/glacial acetic acid/water (150:27:2.5:0.3, v/v/v/v).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Findings:

A confined rotational crop study of ^{14}C -bensulfuron methyl was conducted using Keyport silt loam soil treated with either [phenyl(U)- ^{14}C]bensulfuron methyl or [pyrimidine-2- ^{14}C]bensulfuron methyl at a rate of 67.2 g a.s./ha.

Effect on plants: At the application rate of 67.2 g a.s./ha there was no indication of compound related injury to the crops.

Characterisation of radiolabeled residue in soil samples: The total radioactive residues (TRR, calculated as mg/kg equivalents of bensulfuron methyl) in various parts of all rotational crops were <0.01 mg/kg as shown in the Table B.7. 31. Therefore, no further analysis of the crop fractions was conducted.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Table B.7. 31: The total ^{14}C residues in raw agricultural commodities from the rotational crops sampled at various intervals after soil application with ^{14}C bensulfuron methyl

		^{14}C bensulfuron methyl equivalent residues (mg/kg) ^a									
		[Phenyl(U)- ^{14}C]bensulfuron methyl (days after last application)					[Pyrimidine-2- ^{14}C] bensulfuron methyl (days after last application)				
Crop	Sample	30	45	60	75	105	30	45	60	75	105
Wheat	Stalk	0.008	0.006	0.005	0.005	0.008	0.006	0.005	0.006	0.004	≤0.010
	Grain	NA	NA	0.003	0.005	≤0.010	NA	NA	0.003	0.004	0.009
Cabbage ^b	Foliage	NA	0.002	<0.001	0.002	<0.001	NA	0.002	<0.001	<0.001	<0.001
Green onions ^c	Foliage	0.002	0.002	0.002	0.001	0.001	0.002	0.002	0.002	0.001	0.002
	Bulb	0.001	0.002	0.001	0.001	<0.001	0.001	0.003	0.002	<0.001	<0.001
Red onions ^d	Foliage	0.002	0.003	0.002	NA	0.004	0.002	0.002	0.002	NA	0.002
	Bulb	<0.001	0.001	<0.001	NA	≤0.001	0.002	<0.001	0.002	NA	0.001
Garlic	Foliage	0.002	0.002	0.001	NA	0.003	<0.001	<0.001	0.002	NA	0.003
	Bulb	<0.001	<0.001	0.001	NA	<0.001	<0.001	<0.001	<0.001	NA	0.002
Beets	Foliage	0.003	0.004	0.002	NA	0.004	0.002	0.003	<0.001	NA	0.002
	Root	NA	NA	NA	NA	<0.001	NA	NA	NA	NA	0.001
Cabbage ^e	Foliage	NA	NA	NA	NA	0.002	NA	NA	NA	NA	0.002

^a The total radioactive residues in the commodities were negligible and were reported in the original report as parts per billion (ppb). The values were converted to mg/kg (mg/kg) and rounded up to the nearest significant figure (5 and above were rounded to the next higher number where as 1-4 to the lower number.

^b Chinese Cabbage

^c Green onions

^d Red onions

^e Dwarf Cabbage

NA = not available

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Total radiolabeled residue in soil samples: Characterisation of radioactive residues was conducted using the soil samples taken prior to planting (120 days after application) and at harvest. The total radioactive residues (TRR), extracted and unextracted ^{14}C residues, and intact bensulfuron methyl are reported in Table B.7. 32. There were no significant differences in the TRR, extracted, and unextracted ^{14}C residues from the soil samples taken prior to planting and at harvest.

Table B.7. 32: Total ^{14}C residues and ^{14}C bensulfuron methyl in soil prior to planting rotational crops and at harvest

	Soil sample taken prior to planting (120 days after treatment)			
	^{14}C residues, mg/kg ^a			^{14}C bensulfuron methyl
	TRR	Extracted	Unextracted	
[Phenyl(U)- ^{14}C]	0.006	0.005	0.001	0.005
[Pyrimidine-2- ^{14}C]	0.008	0.006	0.002	0.006
Soil sample taken at harvest				
[Phenyl(U)- ^{14}C]	0.005	0.005	0.001	0.005
[Pyrimidine-2- ^{14}C]	0.008	0.008	0.002	0.006

^a The total radioactive residues in the soil were negligible and were reported in the original report as parts per billion (ppb). The values were converted to mg/kg (mg/kg) and rounded up to the significant figure (5 and above were rounded to the next higher number where as 1-4 to the lower number).

Conclusion:

Total ^{14}C residues in the food items from the wheat, onions, garlic, cabbage, and beets grown in the soil treated with ^{14}C bensulfuron methyl at the rate of 67.2 g a.s./ha and aged for 120 days prior to the planting, were less than 0.01 mg/kg.

The total radioactive residues in the soil at the time of planting and at harvest were also ≤ 0.01 mg/kg. The majority of soil radioactivity was extracted. Unextracted radioactivity was < 0.002 mg/kg. Bensulfuron methyl was the only radioactive residue in all soil samples and was present in the 0.005 to 0.006 mg/kg range.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods or storage periods, in the case of post-harvest uses (Annex IIA 6.8; Annex IIIA 8.7)

B.7.10.1 Proposed pre-harvest intervals (PHIs)

Pre-harvest intervals are covered by conditions of use/or growing period between application and harvest. Since the application is made prior to or during early crop development, since exact PHI is determined by rice varietal variations and since residue levels in mature rice grain were below the limit of quantification, it was not necessary to establish a pre-harvest interval in days for bensulfuron methyl.

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIIA 12.2)

EU MRLs for bensulfuron methyl have not been set yet. National MRLs for rice have been set in the following countries: France 0.05 mg/kg; Italy 0.05 mg/kg; Portugal 0.05 mg/kg; Spain 0.05 mg/kg; Hungary 0.02 mg/kg.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)

The MRL proposals refer to the residue definition as proposed in paragraph B.7.3., i.e. the parent compound bensulfuron methyl. Plant and animal metabolism studies firmly establish that bensulfuron methyl is rapidly and extensively metabolised and that the appropriate analyte for crop residue studies is bensulfuron methyl.

Residue studies show that there are no quantifiable residues of bensulfuron methyl in rice grain specimens from supervised field trials for the analytical methods used for specimen analysis (Table B.7. 30). The calculation of the maximum residue values was carried out according to the statistical methods described in the Working Document 7039/VI/95 EN (22/7/1997) of the Commission of the European Communities – Directorate General for Agriculture VI B II-1 as method I and method II. In Table B.7. 33 the highest residues found in supervised residue trials, the STMR, the calculated R_{max} , R_{ber} and MRL are summarised for flooded and saturated conditions separately. Moreover the EU-MRLs are proposed.

For flooded rice residue levels in rice grain at commercial harvest were below the limit of quantification: <LOQ of 0.020 mg/kg for grain specimens harvested during the 1995 and 1996 growing seasons (6 trials) and <LOQ of 0.010 mg/kg for grain samples harvested during the 2000 growing season (3 trials).

Since residue levels in rice grain in saturated soil conditions (4 trials) at commercial harvest were below the limit of quantification (0.010 mg/kg), the MRL for rice grain is proposed at 0.010 mg/kg. Therefore, an EU-MRL (flooded rice) of 0.02 mg/kg is proposed.

Residue levels in rice straw from field trials in flooded conditions were below the limit of quantification (<LOQ of 0.05 mg/kg) for specimens harvested during the 2000 growing season (3 trials). In saturated soil conditions residue levels were below the limit of quantification of 0.05 mg/kg for specimens harvested during the 2002 growing season (3 trials), except for a residue value of 0.1 mg/kg harvested from the field trial in Spain [report Dupont-9208, *cross reference* 6.3/05].

Table B.7. 33: Summary of found residues and proposed EU MRLs for bensulfuron in rice grain and rice straw

Crop	Field conditions	Highest residue	STMR	R_{max}	R_{ber}	MRL	Proposed EU-MRL
Rice grain	Flooded	0.02 mg/kg	0.02 mg/kg	0.0318mg/kg	0.04 mg/kg	0.02 mg/kg	0.02 mg/kg
	Saturated	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg	0.02 mg/kg	0.01 mg/kg	
Rice straw	Flooded	0.05 mg/kg	0.05 mg/kg	0.05 mg/kg	0.1 mg/kg	0.05 mg/kg	0.1 mg/kg
	Saturated	0.1 mg/kg	0.05 mg/kg	0.19 mg/kg	0.17 mg/kg	0.1 mg/kg	

B.7.13 Proposed EU import tolerances and justification for acceptability of those residues(Annex IIA 6.7 and IIIA 8.6)

No import tolerance has been submitted.

B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

No CAC MRLs have been established or proposed.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.7.15.1 Chronic exposure through diet

The calculation of the potential intake of bensulfuron residues in rice is made based on the proposed MRLs of 0.02 mg/kg as proposed in point B.7.12. Estimation of dietary exposures of populations to residues of bensulfuron methyl has been made as the Theoretical Maximum Daily Intake (TMDI) and compared to the ADI (0.2 mg/kg bw/day) proposed in chapter B.6. Estimation of dietary exposure for a population requires food consumption data and knowledge of the pesticide residues contained in each dietary component. In the absence of actual residue data on the food as consumed, the proposed maximum residue limit (MRL) for rice is used to estimate the TMDI. For this theoretical estimate, following conservative assumptions are utilised:

- 100% of the crop is treated
- Residues are present at the proposed MRL (0.02 mg/kg)
- As residues of bensulfuron methyl in food commodities at harvest are <0.1 mg/kg, it was not necessary to conduct processing studies. Therefore, no cooking/processing correction factors are used in the calculation.

As animal metabolism studies have shown that no detectable residues are present in foodstuffs of animal origin, these products have not been included in the dietary risk assessment.

Different diets and models were used to estimate the long-term dietary intake of bensulfuron methyl residues. The TMDI of the WHO/FAO and German model is based on mean consumption data whereas the TMDI of the UK model is calculated using the two highest contribution commodities at 97.5th percentile consumption and other commodities at the mean.

Following the “Guidelines for predicting dietary intake of pesticide residues” (2nd revised edition, WHO/FSF/FOS/97.7, Geneva 1997), the TMDI was calculated on the basis of both the consumption data given by the European Diet (GEMS/FOOD Regional Diets, WHO, 2003) and the Consumption Cluster Diets as published in January 2006 (Table B.7. 34).

Table B.7. 34: Calculation of the theoretical maximum daily intake (TMDI) on the basis of the European diet and Consumption Cluster Diets (WHO)

Diet/model	Person	Body weight (kg)	Mean rice consumption (kg/person/day)	Proposed MRL on rice (mg/kg)	TMDI (µg/kg bw/day)	% of ADI
WHO/FAO European ^a	Adult	60	0.0130	0.02	0.004	<0.1
WHO/FAO Consumption Cluster Diets ^b						
Cluster B	Adult	60	0.0320	0.02	0.011	<0.1
Cluster E	Adult	60	0.0130	0.02	0.004	<0.1
Cluster F	Adult	60	0.0130	0.02	0.004	<0.1
Cluster D	Adult	60	0.0330	0.02	0.011	<0.1

^aWHO/GEMS FAO European diet, WHO/FSF/FOS/98.3

^bWHO/GEMS FAO, January 2006. (Cluster B : Cyprus, Greece, Italy, Portugal, Spain, Turkey, Cluster E: Austria, Belgium, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Malta, Netherlands, Poland, Slovakia, Slovenia, Switzerland, United Kingdom, Yugoslavia, Cluster F: Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Sweden, Cluster D: Bulgaria, Romania.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

The calculation of the chronic exposure according to the UK Guidance on the Estimation of Dietary Intakes of Pesticides (October 2005) is based on the 97.5th percentile consumption data for adults (76 kg), infants (8.7 kg), toddlers (14.5 kg), 4-6 years-old children (20.5 kg), 7-10 years-old children (30.9 kg), 11-14 years-old children (48 kg), 15-18 years-old children, vegetarians (66.7 kg) and elderly people (living in their own home, 70.8 kg; residential, 61.6 kg). The calculation of TMDIs in the UK model was performed based on the model version 1.1 released on the 23rd January 2006 (Table B.7. 35).

Table B.7. 35: Calculation of the theoretical maximum daily intake (TMDI) according to the UK Consumer Exposure Model

Person	Body weight (kg)	Mean rice consumption (kg/person/day)	High food consumption 97.5 th percentile (kg/person/day)	Proposed MRL on rice (mg/kg)	TMDI (µg/kg bw/day)	% of ADI
Adult	76	0.0278	0.1716	0.02	0.045	<1
Infant	8.7	0.0055	0.0244	0.02	0.056	<1
Toddler	14.5	0.0084	0.0687	0.02	0.094	<1
4-6 Years	20.5	0.0136	0.0761	0.02	0.074	<1
7-10 Years	30.9	0.0203	0.1567	0.02	0.10	<1
11-14 Years	48	0.0294	0.1886	0.02	0.078	<1
15-18 Years	63.8	0.0320	0.1698	0.02	0.053	<1
Vegetarian	66.7	0.0255	0.1338	0.02	0.04	<1
Elderly (Own home)	70.8	0.0065	0.0843	0.02	0.023	<1
Elderly (Residential)	61.6	0.0024	0.0300	0.02	0.0097	<1

PSD Data Requirements Handbook, Chapter 5: Residues

The calculation of the chronic exposure with the German model was performed for children 2-5 years old (16.15 kg bw) according to the BfR (Bundesinstitut für Risikobewertung – German Federal Institute for Risk Assessment) model of May 2005 (Table B.7. 36).

Table B.7. 36: Calculation of the theoretical maximum daily intake (TMDI) according to the German model

Person	Body weight (kg)	Mean rice consumption (kg/person/day)	Proposed MRL on rice (mg/kg)	TMDI (µg/kg bw/day)	% of ADI
Child 2-5 years	16.15	0.0043	0.02	0.005	<0.1

Banasiak U, Hesecker H, Sieke C, Sommerfeld C, Vohmann C (2005). Abschätzung der Aufnahme von Pflanzenschutzmittel-Rückständen in der Nahrung mit neuen Verzehrsmengen für Kinder. (Estimation of the dietary intake of pesticide residues based on new consumption data for children). Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 2005 48:84-98

In conclusion, the highest TMDI calculated with all models is 0.1 µg/kg bw/day which indicates less than 1% of the ADI (0.2 mg/kg bw/day) is accounted for and hence it is not necessary to provide a more refined calculation. As evident, it is clear that the dietary intake of the residue of concern will be below the ADI.

B.7.15.2 Short-term dietary risk assessment

No Acute Reference Dose (ARfD) needs to be set (see B.6.). Acute exposure to bensulfuron methyl residues in food is not likely to cause any unacceptable risk to consumers. It is not necessary to perform IESTI/NESTI calculations.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

B.7.15.3 Overall conclusions on dietary exposure

Long-term dietary risk assessment: The highest TMDI is 0.1 µg/kg bw/day (UK Consumer Exposure Model, 7-10 years old children), which indicates less than 1% of the ADI (0.2 mg/kg bw/day) is accounted for and hence it is not necessary to provide a more refined calculation. No chronic risk to the consumer is to be expected by way of residue intake with food.

Short-Term dietary risk assessment: no Acute Reference Dose (ARfD) needs to be set (see B.6). Acute exposure to bensulfuron methyl residues in food is not likely to cause any unacceptable risk to consumers. It is not necessary to perform IESTI/NESTI calculations.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)**B.7.16.1 Plant metabolism**

The metabolism of ¹⁴C bensulfuron methyl has been studied in rice plants. Rice paddies were treated with one application of [phenyl(U)-¹⁴C]bensulfuron methyl or [pyrimidine-2-¹⁴C]bensulfuron methyl. Rice was grown in miniature rice paddies maintained outside and treated with a single application at 90 g a.s./ha (normal maximum use rate is 60 g a.s./ha). The ¹⁴C residue levels in rice foliage and grain were 0.009 and 0.003 mg/kg, respectively. Metabolites observed in the foliage were *O*-demethylated metabolite (IN-F7880) and the sulfonylurea bridge cleavage products sulfonamide (IN-N5297) and pyrimidine amine (IN-J0290). The sulfonamide cyclises to yield homo-saccharin (IN-B6895). Bensulfuron methyl rice plant metabolism studies demonstrated that bensulfuron methyl was the only relevant plant residue to be determined in mature rice grain.

The proposed residue definition for plants is bensulfuron methyl for monitoring and risk assessment purposes.

B.7.16.2 Livestock metabolism

According to European guidelines in Appendix F doc. 7030/VI/95 rev 3 (22/7/1997) since the dietary animal burden proved to be well below the trigger value of 0.1 mg/kg total diet. One study in goat was submitted and evaluated as additional information (only one ring labelled). Another study in laying hens was submitted and evaluated as additional information (both rings labelled). Results showed a minimal transfer of bensulfuron methyl equivalent residues to the eggs, milk, fat, and tissues and organs of livestock was observed following administration of ¹⁴C bensulfuron methyl to the goat and laying hen. There was minimal potential for bioaccumulation of bensulfuron methyl in the tissues. Most of the dosed radioactivity (greater than 73%) was rapidly excreted from the livestock (goat and poultry) within 48 hours of the administered dose.

Approximately 50 mg per dose (including 47 mg of non-radioactive bensulfuron methyl) was administered to the goat for 7 consecutive days. Essentially, the entire administered radioactivity was recovered in the urine (approximately 52%) and faeces (approximately 49%). The total ¹⁴C residues found in various organs and tissues in the goat, with the exception of liver tissue, were all below the level of detection (0.3 mg/kg). The residue level found in the liver tissue was 0.4 mg/kg. A very low level was found in milk (0.018 to 0.031 mg/kg). Analysis of the milk indicated the presence of six metabolites together with a trace amount of bensulfuron methyl (≤0.002 mg/kg).

The laying hen was dosed orally with a capsule containing approximately 0.013 mg ¹⁴C bensulfuron methyl for 14 consecutive days. Elimination of dosed radioactivity was rapid and approximately 80% in laying hen. Bensulfuron methyl residues in the hen, eggs, blood, and tissues, except the GI tract, were below the detection limit (0.002 mg/kg). No radioactivity was detected in the eggs; only the GI tract and liver had ¹⁴C residue levels at or above

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

0.01% (0.1% in the liver and 0.6-0.8% in the GI tract). The total ^{14}C residue levels in the GI tract were between 0.012 and 0.014 mg/kg. Even when the hen was administered with the high dose (500 times higher than the low dose), the tissue radioactivity concentration was not significant.

The major component in animal excreta was unchanged bensulfuron methyl. The primary pathway was *O*-desmethylation of the pyrimidine ring yielding *O*-desmethyl-bensulfuron methyl (IN-F7880). A second metabolic pathway involved hydroxylation of the phenyl ring to form IN-W8956. Another major metabolite was formed in the goat by hydroxylation of the pyrimidine ring to yield IN-N8989. A minor metabolic pathway in the goat involved cleavage of the sulfonylurea bridge to form the sulphonamide (IN-N5297). A minor pathway observed in the hen involved hydroxylation of the phenyl ring to yield IN-W8956.

The proposed residue definition for animals is bensulfuron methyl for monitoring and risk assessment purposes.

B.7.16.3 Residues resulting from supervised trials

Trials to meet EU requirements according to or exceeding GAP requirements were carried out in flooded and saturated land rice with formulations containing bensulfuron methyl. The magnitude of bensulfuron methyl residues was monitored in a total of 14 field residue trials, representing pre-harvest uses on rice: 10 trials on flooded rice and 4 trials on saturated land rice. These trials were conducted over five growing seasons, 1991, 1995, 1996, 2000, and 2002 in locations representative of major rice production areas in the southern European region, using representative Bensulfuron methyl WG formulations. Eight residue trials were conducted using Bensulfuron methyl 60WG; six residue trials were conducted using DPX-JM206 60WG (contained 58% a.s. bensulfuron methyl + 2% a.s. metsulfuron methyl). The trials covered a wide range of locations, climates and soil types within southern Europe and the data obtained reflect the commercial use of the product in the major crop growing regions.

The GAP for use of bensulfuron methyl on flooded or saturated land rice specifies application of up to 60 g a.s./ha as late as growth stage 29 (BBCH 29, end of tillering). Application timing must not produce a PHI shorter than 60 days. When bensulfuron methyl is applied with timing specified by GAP, there are no quantifiable residues in rice grain. The likelihood of no residues in rice grain at normal harvest is further strengthened by evidence of no quantifiable residue in rice grain when applications are made at timing slightly beyond the BBCH 29 growth stage (BBCH 32). Residues in rice grain were consistently below the LOQ of the analytical methods used for residue analysis [0.02 or 0.01 mg/kg] for all residue field trials. All residues were determined using validated methods, which produced good recovery data for fortified control specimens run concurrently with treated specimens.

Since the study conducted in Greece in 1991 (*cross reference IIA, 6.3/03*) was evaluated not acceptable, the residue data from it are not used for the calculation of MRL. The residue data from grain field trials performed in compliance with the GAP are sufficient for the calculation of a MRL of 0.02 mg/kg to be proposed for rice grain.

B.7.16.4 Feeding studies in livestock

According to the European guidelines in Appendix G doc. 7031/VI/95 rev.4 (22/7/96) livestock feeding studies are required when significant residues (≥ 0.1 mg/kg of the total diet received) occur in crops fed to animals. In addition, livestock feeding studies are required when metabolism studies indicate that significant residues (>0.1 mg/kg) may occur in products of animal origin as a result of animals consuming feeding stuffs or fodder crops containing residues. In the case of bensulfuron methyl, livestock feeding studies are not required since anticipated residues in the diet as received are less than 0.1 mg/kg. There are no quantifiable residues of the active substance in mature rice

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

grain at harvest as shown in the plant metabolism studies and crop residue studies. Feeding studies are not necessary to assess human exposure to bensulfuron methyl from animal food sources.

B.7.16.5 Effects of industrial processing and/or household preparation on the nature and magnitude of residues

According to the European guidelines in Appendix E doc. 7035/VI/95 rev.5 (22/7/97) processing studies are not necessary “if no significant (>0.1 mg/kg) or no analytically quantifiable residues occur in the plant product being processed”. No quantifiable bensulfuron methyl residues were found in any mature rice grain specimen at the time of harvest. Therefore, no processing study is necessary.

B.7.16.6 Residue data in succeeding or rotational crops

The total radioactive residues in the [phenyl(^{14}C)]bensulfuron methyl and [pyrimidine-2- ^{14}C)]bensulfuron methyl treated soils at planting were <0.01 mg/kg. The radioactivity residues did not accumulate in rotational crops grown in Keyport silt-loam soil treated with ^{14}C bensulfuron methyl at the rate of 67.2 g a.s./ha and aged for 120 days prior to the planting. The majority of soil radioactivity was extracted. Unextracted radioactivity was <0.002 mg/kg. Bensulfuron methyl was the only radioactive residue in soil samples and was present at 0.005-0.006 mg/kg. Total ^{14}C residues in food items from wheat, onions, garlic, cabbage, and beets grown in the soil treated were less than 0.01 mg/kg. Since all plant samples contained less than 0.01 mg/kg (10 $\mu\text{g/kg}$) total ^{14}C residues, further work to characterise radiolabeled metabolites was not attempted.

B.7.16.7 Proposed maximum residue levels (MRLs)

For flooded rice residue levels in rice grain at commercial harvest were below the limit of quantification: $<\text{LOQ}$ of 0.020 mg/kg for grain specimens harvested during the 1995 and 1996 growing seasons (6 trials) and $<\text{LOQ}$ of 0.010 mg/kg for grain samples harvested during the 2000 growing season (3 trials). The MRL value for flooded rice is calculated to be 0.020 mg/kg. Since residue levels in rice grain in saturated soil conditions at commercial harvest were below the limit of quantification (0.010 mg/kg; 4 trials), the MRL for rice grain is proposed at 0.01 mg/kg, which is the limit of quantification of the proposed MRL monitoring method. Therefore, an EU-MRL (estimated as the worst case) of 0.02 mg/kg is proposed for rice grain. Residue levels in rice straw from field trials in flooded conditions were below the limit of quantification ($<\text{LOQ}$ of 0.05 mg/kg) for specimens harvested during the 2000 growing season (3 trials). In saturated soil conditions residue levels were below the limit of quantification of 0.05 mg/kg for specimens harvested during the 2002 growing season (3 trials), except for a residue value of 0.1 mg/kg harvested from the field trial in Spain.

B.7.16.8 Proposed pre-harvest intervals for envisaged uses

Pre-harvest intervals are covered by conditions of use/or growing period between application and harvest. Since the application is made prior to or during early crop development, since exact PHI is determined by rice varietal variations and since residue levels in mature rice grain were below the limit of quantification, it was not necessary to establish a pre-harvest interval in days for bensulfuron methyl.

B.7.16.9 Dietary exposure

The calculation of the potential intake of bensulfuron residues in rice is made based on the proposed MRLs of 0.02 mg/kg as proposed in point B.7.12. Estimation of dietary exposures of populations to residues of bensulfuron methyl

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

has been made as the Theoretical Maximum Daily Intake (TMDI) and compared to the ADI (0.2 mg/kg bw/day) established in chapter B.6.

As residues of bensulfuron methyl in food commodities at harvest are <0.1 mg/kg it was not necessary to conduct processing studies. Therefore, no cooking/processing correction factors are used in the calculation.

As animal metabolism studies have shown that no detectable residues are present in foodstuffs of animal origin, these products have not been included in the dietary risk assessment.

The highest TMDI is 0.1 µg/kg bw/day (UK Consumer Exposure Model, 7-10 years old children), which indicates less than 0.1% of the ADI (0.2 mg/kg bw/day) is accounted for and hence it is not necessary to provide a more refined calculation. In conclusion, the highest TMDI calculated with all models is 0.1 µg/kg bw/day which indicates less than 1% of the ADI (0.2 mg/kg bw/day) is accounted for and hence it is not necessary to provide a more refined calculation. As evident, it is clear that the dietary intake of the residue of concern will be below the ADI. No chronic risk to the consumer is to be expected by way of residue intake with food.

No Acute Reference Dose (ARfD) has been established because toxicological end points are not relevant for acute oral dietary exposure; consequently no acute dietary exposure has been estimated.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl
Residue data		

B.7.17 References relied on

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 6.0./01	Devine, T. J.	1997	Freezer storage stability of DPX-F5384 in rice straw samples DuPont Experimental Station AMR 3056-94 GLP: Yes Published: No	Y	DuPont
IIA, 6.0./02	Nathan III, E.C.	2002	Storage stability of bensulfuron methyl in rice grain: Data summary in support of the registration of Londax® 60 DF on rice crops in Southern Europe DuPont Stine-Haskell Research Center DuPont-8797 GLP: No Published: No	Y	DuPont
IIA, 6.0./03	Weber, H., Bramble Jr, F.Q.	2002	Magnitude of residues of bensulfuron methyl in rice (cereals) following application of bensulfuron methyl 60 WG formulation in paddy rice fields - southern europe, season 2000 Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-4139 GLP: Yes Published: No	Y	DuPont
IIA, 6.1./01	Hunt, O.R., Berus, J.S.	1986	Metabolism of [Phenyl(U)-14C] DPX-F5384 and [Pyrimidine-2-14C] DPX-F5384 in Field-Grown Japonica Rice DuPont Experimental Station AMR 473-86 GLP: No Published: No	N	DuPont
IIA, 6.2./01	Hunt, O. R.	1985	Metabolism of [phenyl- ¹⁴ C(U)] DPX-F5384 in a lactating goat [REDACTED] AMR 219-84, Revision No. 1 GLP: No Published: No	N	DuPont
IIA, 6.2./02	Scott, M.T.	1991	Metabolism study of [¹⁴ C]bensulfuron methyl in laying hens [REDACTED] AMR 1454-89 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Residue data

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 6.3./01	Bonacini, F., Zenide, D., Gasser, F., Leka, A.	1998	Field dissipation of bensulfuron methyl and metsulfuron methyl from a representative rice field following treatment of three consecutive years with Sigma 60 DF herbicide in Italy Battelle Europe - Geneva Research Centres AMR 4162-96 GLP: Yes Published: No	Y	DuPont
IIA, 6.3./02	Bonacini, F., Zenide, D., Gasser, F., Leka, A.	1999	Field dissipation of bensulfuron methyl and metsulfuron methyl from a representative rice field following treatment of three consecutive years with Sigma 60 DF herbicide in Italy seasons 1994-1996, and magnitude of residue in rice grains Battelle Europe - Geneva Research Centres AMR 4162-96, Amendment No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 6.3./04	Weber, H., Bramble Jr, F.Q.	2002	Magnitude of residues of bensulfuron methyl in rice (cereals) following application of bensulfuron methyl 60 WG formulation in paddy rice fields - Southern Europe, Season 2000 Dr. Specht & Partner Chemische Laboratorien GmbH DuPont-4139 GLP: Yes Published: No	Y	DuPont
IIA, 6.3./05	Weber, H., Hornshuh, M.J.	2003	Magnitude of residues of bensulfuron methyl in rice (cereals) following application of bensulfuron methyl (DPX-F5384) 60WG formulation in wetland rice fields - Southern Europe, Season 2002 DuPont Experimental Station DuPont-9208 GLP: Yes Published: No	Y	DuPont
IIA, 6.6./01	Monson, K.D., Bullock, J.H.	1985	Crop rotation studies with ¹⁴ C-labeled DPX-F5384 in the greenhouse DuPont Experimental Station AMR 335-85 GLP: No Published: No	N	DuPont