

ANNEX B

Active substance: Bensulfuron methyl

B.6 Toxicology and metabolism

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.

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B.6. Toxicology and metabolism

This document contains summaries of toxicity tests completed for Bensulfuron methyl and overviews by groups of tests. Test summaries cover the individual study types and a discussion of the primary toxicological effects.

Test substance specification can be determined from the test substance code which is a research and development code number given to a specific batch of produced material (either technical or formulated). The approximate composition of the materials used in the various tests are given in Table B.6.1.

Table B.6.1 Test substance specification

Test Substance Code	Type	Purity (%)	Specification
DPX-F5384-52	Active substance as manufactured	95.9	See Document J
DPX-F5384-38	Active substance as manufactured	94	See Document J
DPX-F5384-122	Active substance as manufactured	96.1	See Document J
DPX-F5384-44	Active substance as manufactured	95	See Document J
DPX-F5384-15	Active substance as manufactured	95.7	See Document J
DPX-F5384-17	Active substance as manufactured	98.5	See Document J
DPX-F5384-18	Active substance as manufactured	98.4	See Document J
DPX-F5384-7	Active substance as manufactured	95	See Document J
DPX-F5384-38	Active substance as manufactured	95.9	See Document J
DPX-F5384-80	Active substance as manufactured	99	See Document J
IN-J0290-1	Technical metabolite	95	See Purity
IN-B6895	Technical metabolite	99.4	See Purity
IN-R9419-1	Technical metabolite	95	See Purity
IN-N5297-1	Technical metabolite	95	See Purity

The detailed composition and impurity profiles of the test substances used in the toxicological studies are provided in Document J.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

B.6.1.1 Metabolism study - rats

- 1) (^{14}C)-Bensulfuron methyl: Absorption, distribution, metabolism and excretion in the rat. Cornelissen, K.S., Lewis, C., Hopkins, R., 1990.

Cross reference IIA, 5.1/01

Guidelines:

USEPA 85-1, 59 NohSan No. 4200

Deviations: None

GLP:

Yes

The study is *acceptable*

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Material and methods

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

Batch: [Phenyl (U)- ^{14}C], [Pyrimidine-2- ^{14}C], F5384-83

Purity: >95%, >95%, 99.5%

- 2) (^{14}C)-Bensulfuron methyl: Absorption, distribution, metabolism and excretion in the rat. Cornelissen, K.S., Lewis, C., Hopkins, R., 1992.

Cross reference IIA, 5.1./02

Guidelines:

USEPA 85-1, 59 NohSan No. 4200

Deviations: Not applicable

The study is *acceptable*

GLP:

Yes

Material and methods

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

Batch: [Phenyl (U)- ^{14}C], [Pyrimidine-2- ^{14}C], F5384-83

Purity: >95%, >95%, 99.5%

- 3) (^{14}C)-Bensulfuron methyl: Absorption, distribution, metabolism and excretion in the rat. Cornelissen, K.S., Lewis, C., Hopkins, R., 1993.

Cross reference IIA, 5.1./03

Guidelines:

USEPA 85-1, 59 NohSan No. 4200

Deviations: Not applicable

GLP:

Yes

The study is *acceptable*

Materials and Methods:

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

Batch: [Phenyl (U)- ^{14}C], [Pyrimidine-2- ^{14}C], F5384-83

Purity: >95%, >95%,

99.5%

Experiments were conducted in male and female Sprague Dawley rats (CrI:(SD)CD BR) using five rats per sex per group and two radiolabels: [phenyl(U)- ^{14}C]bensulfuron methyl or [pyrimidine-2- ^{14}C]bensulfuron methyl. The low and high dose solutions contained enough radiolabel to deliver approximately 80-100 μCi per subject by single oral gavage administration using acetone:corn oil (3:7 v/v) as the dose vehicle. End points evaluated included the pharmacokinetic behaviour of radioactive residues in plasma; the disposition and material balance of total radioactive

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residues among tissues, urine and faeces and expired air; the elimination of radioactive residues in bile; and identification of metabolites in plasma, bile, urine, and faeces. The dose groups and number of animals used were as follow:

Table B.6.2: Study design for metabolism of ^{14}C -bensulfuron methyl in rats

CrI:(SD)CD BR Dose group	Number of rats for oral gavage administration					
	Pharmacokinetic ^a		Disposition and Material balance ^b		Tissue distribution ^c	
	Male	Female	Male	Female	Male	Female
A	5	5	5	5	15	15
B	NA	NA	5	5	NA	NA
C	5	5	5	5	15	15
D	5	5	5	5	15	20
E	NA	NA	5 ^d	5 ^d	NA	NA

Group A: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group B: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl, following 15 days of oral gavage pre-treatment with unlabeled bensulfuron methyl

Group C: 1000 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group D: 1000 mg/kg bw [pyrimidine-2- ^{14}C]bensulfuron methyl

Group E: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl with cannulation of bile duct.

Samples collected for ^{14}C analysis included:

^a Whole blood and plasma (0, 1, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h), urine and faeces (0-6, 6-12, 12-24, 24-48, 48-72 and 72-96 h), and cage wash (at 96 h).

^b Excreta (0-6, 6-12, 12-24, 24-48, 48-72, and 72-96 h), cage wash (96 hours), expired air (0-12, 12-24 h), and residual diet and eighteen tissues (96 h), except for Group E.

^c Tissues corresponding to C_{max} and $C_{\text{max}}/2$ were collected at 1, 4 and 12h (except Group D female which included an additional collection at 24 h).

^d Group E bile (0-1, 1-2, 2-4, 4-6, 6-12, and 12-24 h) and urine and faeces (0-12 and 12-24 h).

Findings:

Pharmacokinetics

The pharmacokinetics of ^{14}C residues in plasma demonstrated that absorption occurred within 1-4 hours after low dose administration (20 mg/kg bw), and 4-24 hours after high dose administration (1000 mg/kg bw) (Table B.6. 3). The pharmacokinetic analysis for plasma gave a time to C_{max} ($t_{C_{\text{max}}}$) of 1 hour after low dose administration and 6 to 13 hours after high dose administration (Table B.6. 3). The C_{max} and AUC values did not increase proportionately with dose. For example, the 50-fold increase in dose resulted in approximately a 30-fold higher AUC, indicating saturation of absorption at the higher dose. The elimination half-lives ($t_{1/2}$) for total radioactive residues in plasma ranged from 5.3 to 9.1 hours independent of sex and dose (Table B.6. 3). Radioactivity in plasma was below detection by 72 hours after dosing. The time course of radioactivity in blood was similar to that measured in plasma except the C_{max} concentrations were approximately 50% lower, indicating no apparent binding to red blood cells.

Disposition and Material Balance

The results of the disposition and material balance experiment are summarised in Table B.6. 4. The elimination of radioactivity was essentially complete 48-72 hours after oral administration of ^{14}C bensulfuron methyl. No apparent sex differences in the excretion pattern were observed in any of the groups, regardless of the position of ^{14}C -label. No radioactivity was detected in expired breath. A greater proportion of the dose was excreted in the faeces after high dose administration (1000 mg/kg bw) most likely because of reduced gastrointestinal tract absorption. Pre-treatment with bensulfuron methyl for 15 days (Group B) at the low dose level (20 mg/kg bw) had no major effect on the route

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and rate of elimination when compared with single dose oral gavage administration of ^{14}C bensulfuron methyl at the same dose level.

Tissue Residues

The majority of retained (un-excreted) radioactivity as a percentage of administered dose was recovered from the gastrointestinal tract (GIT) contents, GI tissue, residual carcass, and liver. Less than 0.3% of the dose remained in all tissues and carcass by 96h after dose administration. The concentration of ^{14}C -residues was greatest in the GIT contents >GIT tissue >liver >kidney >plasma. An increase in dose level from 20 to 1000 mg/kg bw was found to prolong the time to reach peak tissue concentration from 1 to 4 hours post-dose, consistent with the delay observed in the plasma pharmacokinetic data. No statistically significant sex differences were observed in the tissue distribution, with the exception that the position of ^{14}C -label appeared to affect the plasma $t_{\text{C}_{\text{max}}}$. In this instance, the [phenyl(U)- ^{14}C]bensulfuron methyl (1000 mg/kg bw, Group C) rats had peak **blood** concentrations typically at 4 hours, whereas the [pyrimidine-2- ^{14}C]bensulfuron methyl (1000 mg/kg bw, Group D) rats had peak concentrations at 4 hours for males and 12 hours for females (Table B.6. 3). Despite this apparent shift in $t_{\text{C}_{\text{max}}}$, the concentrations of radioactivity in the plasma and other tissues were similar for both radiolabels. Tissue concentrations of radioactivity declined markedly between 1 and 96 hours after low or high dose administration with either label. Excluding the GIT contents and GI tissue, tissue-to-plasma ratios were generally less than 1.44 by 12 or 24 hours after low or high dose administration, indicating minimal potential for tissue accumulation. The tissue-to-plasma ratios ranged from 2 to 5 at the earlier time points of 1 and 4 hours. Tissue-to-plasma ratios were not estimated for the 96-hour data because of the low (non-detectable) amounts of ^{14}C -residues in plasma and other tissues.

Biliary Elimination

Biliary excretion was a major route of elimination into the faeces. By 24 hours after 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl (Group E) administration, 29.3 and 15.7% of the dose was recovered in the bile of male and female rats, respectively. Because bile duct cannulation altered normal intestinal function, a reduced percentage of the dose was recovered in faeces compared with the non-cannulated animals (Group A, Table B.6. 4). Because of this, Group E animals were not utilised for determinations of overall gastrointestinal tract absorption. Rather, the non-cannulated animals administered 20 mg/kg bw, Group A, were utilised for this purpose. Faecal radioactivity consists of contributions from the bile and non-absorbed bensulfuron methyl in the intestinal tract. Therefore, the bile excretion data from Group E was used to estimate the contribution of bile to the faecal radioactivity for Group A. In bile duct cannulated male and female rats, the percentage of dose excreted in the bile after 24 hours was 29.3 and 15.7% for male and female rats, respectively (Table B.6. 4). Since the absorption process is not complete in 24 hours (*i.e.*, the cumulative excretion pharmacokinetics for bensulfuron methyl Group A plateaus at approximately 48 hours), the percentages of excretion into the bile are expected to further increase. When these 24-hour bile contributions were added to the radioactivity recovered from the urine, cage wash and tissues, the gastrointestinal tract absorption of bensulfuron methyl was estimated at >90 and >83% for males and females, respectively (Table B.6. 5). These represent minimal values for the following reasons: the absorption and biliary excretion process is not complete at 24 hours, and there were no corrections for percent recovery of ^{14}C -residues. The presence of low quantities of unmetabolised bensulfuron methyl in excreta at this dose (<5% based on Group B data; Table B.6. 6) further supports a higher value for gastrointestinal tract absorption.

Metabolites (Figure B.6. 1)

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Radioactive residues were quantitatively extracted from urine, faeces, bile, and plasma and analysed by TLC (thin layer chromatography). The metabolic profile and quantities were independent of sex and time point. Pre-treatment with non-radiolabeled bensulfuron methyl had no effect upon the metabolite profiles in either urine or faeces. Faeces, urine, and plasma profiles were independent of dose level.

Plasma: At $t_{C_{max}}$ (1 hour), bensulfuron methyl (10.7-13.0 $\mu\text{g/g}$ plasma) was the major radioactive component in the plasma of rats administered 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl (Group A). *O*-demethyl bensulfuron methyl (IN-F7880) was observed at a concentration of 0.6-1.1 $\mu\text{g/g}$ in the 1-hour sample. Bensulfuron (IN-R9419) was present at a lower concentration of 0.3 $\mu\text{g/g}$ plasma. Homosaccharin (IN-B6895) was present in plasma at low concentrations ranging from not detectable to 1.5 $\mu\text{g/g}$ plasma between 1 and 12 hours after high dose administration of [phenyl(U)- ^{14}C]bensulfuron methyl (1000 mg/kg bw, Group C). Bensulfuron methyl, IN-F7880, and IN-R9419 were also identified in plasma after high dose administration of [pyrimidine-2- ^{14}C]bensulfuron methyl (Group D).

Bile: Bile collected from rats administered 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl (Group E) contained two major metabolites. IN-F7880 accounted for 13.7 and 8.3% of the administered dose in the 0-24 hours bile samples of male and female rats, respectively. A polar metabolite accounted for 11.0 and 5.5% in the bile of male and female rats, respectively.

Faeces: Table B.6. 6 summarises the metabolic profile in faeces as percent of administered dose. Faeces from rats administered the low or high dose of [phenyl(U)- ^{14}C]bensulfuron methyl contained three major components: Bensulfuron methyl, IN-F7880, and IN-JF987. The identification of IN-JF987 may have been an artifact of methanol being used during sample processing. The major components in faeces of rats administered 1000 mg/kg [pyrimidine-2- ^{14}C]bensulfuron methyl (Group D) were parent bensulfuron methyl, and un-cleaved metabolite IN-F7880 and cleaved hydroxy-pyrimidine-bensulfuron methyl (IN-N8989).

Urine: Table B.6. 7 summarises the metabolic profile in urine as percent of administered dose. *O*-demethylation of parent bensulfuron methyl resulted in IN-F7880 as the majority urinary metabolite following low dose administration of [phenyl(U)- ^{14}C]bensulfuron methyl (Group A). Other identified metabolites included IN-R9419, IN-N8989, IN-N5297, and IN-JF987; the latter of which may have been methylated during sample processing with methanol.

The urine from rats administered the high dose of [pyrimidine-2- ^{14}C]bensulfuron methyl (Group D) contained essentially the same metabolites observed in the urine of rats administered [phenyl(U)- ^{14}C], except for the identification of *O*-demethyl pyrimidine amine (IN-H9235) specific for the ^{14}C -labeled pyrimidine ring. A small amount of bensulfuron methyl was also observed in urine of Group D rats (Table B.6. 7).

Conclusion:

Absorption was substantially complete by 24 h after dose administration based on the rise and decline of radioactivity in plasma. Peak plasma concentrations occurred at 1-4 hours after oral gavage administration with a single low dose (20 mg/kg bw) and 4 to 12 h after high dose (1000 mg/kg bw). The estimated percent dose absorbed in the 20 mg/kg bw group was >90 and >83% for males and females, respectively.

The elimination half-lives describing the decline of plasma radioactivity ranged from 5.3 to 9.1 hours. Less than 0.3% of the dose remained in all tissues and carcass by 96h after dose administration. The majority of the absorbed dose was excreted in bile and urine.

Tissue-to-plasma ratios for total radioactivity were <1 for low dose and 2-5 for high dose. Residual radioactivity in plasma was undetectable by 72h after dose administration indicating minimal potential for bioaccumulation.

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Excretion in urine and faeces was essentially complete by 48 to 72h after administration, independent of sex, dose level, radiolabel, or preconditioning with non-radiolabeled bensulfuron methyl. After low dose administration, the percent of dose excreted was 44-58% in urine and 27-42% in faeces. After high dose administration with the phenyl or pyrimidine label, excretion in urine was 22-30% and faeces 57-66% of the dose. Biliary excretion through 24h after dose administration was 29 and 16% in male and female rats, respectively.

Bensulfuron methyl undergoes extensive metabolism in rats primarily by *O*-demethylation and hydroxylation of the pyrimidine ring as well as sulfonamide bridge cleavage. No major changes were observed between the pyrimidine or phenyl labels, sexes, low and high dose groups, or preconditioning by multiple dosing with non-radiolabeled bensulfuron methyl. The metabolic profile was similar in the urine and faeces from the male and female rats of low and high dose groups with IN-F7880, IN-N8989, IN-N5297, IN-H9235, and IN-R9419.

Table B.6. 3: Pharmacokinetic parameters for bensulfuron methyl in rats

Parameter	Plasma ^b		Whole blood ^c	
	Male	Female	Male	Female
Group A ^a : 20 mg/kg BW - [phenyl(U)- ¹⁴ C]bensulfuron methyl				
Elimination half-life (t _{1/2} , hr)	7.0 ± 2.0	9.1 ± 2.5	NA	NA
Area-under-the-curve (AUC, µg/g·hr)	106 ± 51	131 ± 19	NA	NA
Peak time (tC _{max} , hr)	1	1	1	1
Peak concentration (C _{max} , µg/g)	16.8 ± 11.2	14.8 ± 4.4	7.7 ± 5.1	7.0 ± 1.9
Group C ^a : 1000 mg/kg BW - [phenyl(U)- ¹⁴ C]bensulfuron methyl				
Elimination half-life (t _{1/2} , hr)	8.1 ± 0.9	7.5 ± 3.6	NA	NA
Area-under-the-curve (AUC, µg/g·hr)	2925 ± 206	4088 ± 411	NA	NA
Peak time (tC _{max} , hr)	6	9	4	4
Peak concentration (C _{max} , µg/g)	181 ± 29	192 ± 28	99 ± 16	101 ± 18
Group D ^a : 1000 mg/kg BW - [pyrimidine-2- ¹⁴ C]bensulfuron methyl				
Elimination half-life (t _{1/2} , hr)	6.3 ± 2.4	5.3 ± 1.0	NA	NA
Area-under-the-curve (AUC, µg/g·hr)	2857 ± 847	3650 ± 787	NA	NA
Peak time (tC _{max} , hr)	6	13	4	12
Peak concentration (C _{max} , µg/g)	213 ± 32	216 ± 58	112 ± 29	121 ± 38

BW - body weight, NA - not applicable, SD - Standard deviation

^a Plasma pharmacokinetics measured in Groups A, C, and D but not B and E by design. Values reported are means (± SD) for five rats per group

^b Parameters derived by pharmacokinetic analysis

^c Experimental data. Pharmacokinetic parameters t_{1/2} and AUC were not derived whole blood

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Table B.6. 4: Mean disposition and material balance of bensulfuron methyl in rats

Sex	Mean percent of dose ($\pm$ SD) in					
	Urine	Faeces	Cage wash ^a	Tissues & G.I. tract	Bile	Recovery ^b
Group A: 20 mg/kg bw [phenyl(U)- ¹⁴ C]bensulfuron methyl (96 h collection)						
Male	49.9 $\pm$ 13.8	31.6 $\pm$ 9.2	11.1 $\pm$ 5.8	0.1	--	92.8 $\pm$ 1.7
Female	57.8 $\pm$ 10.9	27.3 $\pm$ 5.3	9.1 $\pm$ 5.7	0.3	--	94.5 $\pm$ 2.3
Group B: 20 mg/kg bw [phenyl(U)- ¹⁴ C]bensulfuron methyl (with pre-treatment, 96 h collection)						
Male	47.0 $\pm$ 5.4	39.3 $\pm$ 2.4	7.8 $\pm$ 2.7	0.1	--	94.3 $\pm$ 1.1
Female	44.1 $\pm$ 8.5	41.8 $\pm$ 6.4	10.2 $\pm$ 5.0	<0.1	--	96.2 $\pm$ 2.3
Group C: 1000 mg/kg bw [phenyl(U)- ¹⁴ C]bensulfuron methyl (96 h collection)						
Male	24.8 $\pm$ 4.5	58.2 $\pm$ 4.7	3.8 $\pm$ 1.2	<0.1	--	86.9 $\pm$ 0.6
Female	30.2 $\pm$ 3.4	56.8 $\pm$ 4.7	5.0 $\pm$ 1.7	<0.1	--	92.1 $\pm$ 2.0
Group D: 1000 mg/kg bw [pyrimidine-2- ¹⁴ C]bensulfuron methyl (96 h collection)						
Male	24.0 $\pm$ 3.2	64.3 $\pm$ 5.0	3.1 $\pm$ 1.5	<0.1	--	91.5 $\pm$ 1.0
Female	22.4 $\pm$ 4.9	66.2 $\pm$ 2.8	6.1 $\pm$ 5.1	<0.1	--	94.8 $\pm$ 1.7
Group E: 20 mg/kg bw [phenyl(U)- ¹⁴ C]bensulfuron methyl (24 h collection)						
Male	23.3 $\pm$ 14.8	0.8 $\pm$ 0.4	4.9 $\pm$ 3.4	32.5 $\pm$ 18.4 ^c	29.3 $\pm$ 10.5	90.7 $\pm$ 2.5
Female	14.1 $\pm$ 4.1	1.4 $\pm$ 2.2	6.6 $\pm$ 4.7	51.7 $\pm$ 15.1 ^d	15.7 $\pm$ 5.2	89.4 $\pm$ 4.8

^a The radioactivity from cage wash and cage derbies are combined^b No radioactivity was detected in expired breath^c Dose recovered in gastrointestinal contents was 22.0 $\pm$ 14.7%^d Dose recovered in gastrointestinal contents was 38.8 $\pm$ 14.9%**Table B.6. 5: Gastrointestinal tract absorption of bensulfuron methyl in rats**

	Males (Mean % Dose)	Females (Mean % Dose)
Urine	49.9	57.8
Bile ^a	>29.3	>15.7
Cage Wash	11.1	9.1
Tissues	0.1	0.3
Total % Absorbed	>90.4%	>82.9%

^a Bile excretion data from Group E; administered 20 mg/kg bw and analysed after 24-hours.

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

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

Toxicology and metabolism

Table B.6. 6: Metabolites as percent of administered dose in faeces following oral gavage administration of ^{14}C -bensulfuron methyl to rats

Technical or Metabolite	Group A		Group B		Group C		Group D	
	Male	Female	Male	Female	Male	Female	Male	Female
DPX-F5384	-- ^a	-- ^a	3.8	4.3	16.4	13.3	19.9	23.2
IN-R9419	0.4	0.9	ND ^b	ND ^b	0.0	1.2	-- ^c	-- ^c
IN-F7880	16.7	16.2	8.8	12.1	9.6	11.3	12.9 ^d	21.2 ^d
IN-N8989	ND	ND	0.2	0.4	0.2	0.4	1.8	1.4
IN-N5297	0.2	0.2	0.6	1.3	0.5	0.3	NA	NA
IN-JF987 ^e	3.6	1.5	11.0	10.2	9.4	6.0	NA	NA
IN-H9235	NA	NA	NA	NA	NA	NA	-- ^c	-- ^c
Polar	1.5	1.7	4.8	4.1	2.0	1.5	0.6	0.7
Other ^f	4.5	5.4	6.7	4.1	5.0	8.3	16.2	10.0

Group A: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group B: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl, following 15 days of oral gavage pre-treatment with unlabeled bensulfuron methyl

Group C: 1000 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group D: 1000 mg/kg bw [pyrimidine-2- ^{14}C]bensulfuron methyl

NA: not applicable

^a Bensulfuron methyl not resolved well from IN-F7880 for accurate quantitation.

^b Not detected (<0.05% of dose)

^c Quantitation was not possible because of overlap with other components present in chromatogram.

^d IN-F7880 co-eluted with IN-H9235 and IN-R9419.

^e Identification of IN-JF987 may be an artifact of sample processing due to the use of methanol as a reagent.

^f Comprised of multiple unidentified components designated as "other", "remainder", and/or resolved but uncharacterised chromatographic regions.

Table B.6. 7: Metabolites as percent of administered dose in rat urine following oral gavage administration of ^{14}C -bensulfuron methyl to rats

Technical or Metabolite	Group A		Group B		Group C		Group D	
	Male	Female	Male	Female	Male	Female	Male	Female
DPX-F5384	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1
IN-R9419	1.5	1.8	1.4	1.1	1.0	1.8	0.6	1.7
IN-F7880	20.3	27.9	17.7	19.6	3.5	4.1	8.1	9.9
IN-N8989	2.4	0.7	0.9	0.4	0.4	0.7	0.5	0.2
IN-N5297	1.0	1.4	0.5	1.7	0.2	0.5	NA	NA
IN-JF987 ^a	13.2	7.0	8.9	6.2	3.3	5.1	NA	NA
IN-H9235	NA	NA	NA	NA	NA	NA	1.3	1.7
Polar	4.2	3.2	5.4	3.1	6.3	7.3	NR ^b	NR ^b
Other ^c	2.2	1.8	1.8	1.4	0.3	0.4	7.1	4.8

Group A: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group B: 20 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl, following 15 days of oral gavage pre-treatment with unlabeled bensulfuron methyl

Group C: 1000 mg/kg bw [phenyl(U)- ^{14}C]bensulfuron methyl

Group D: 1000 mg/kg bw [pyrimidine-2- ^{14}C]bensulfuron methyl

NA Not applicable

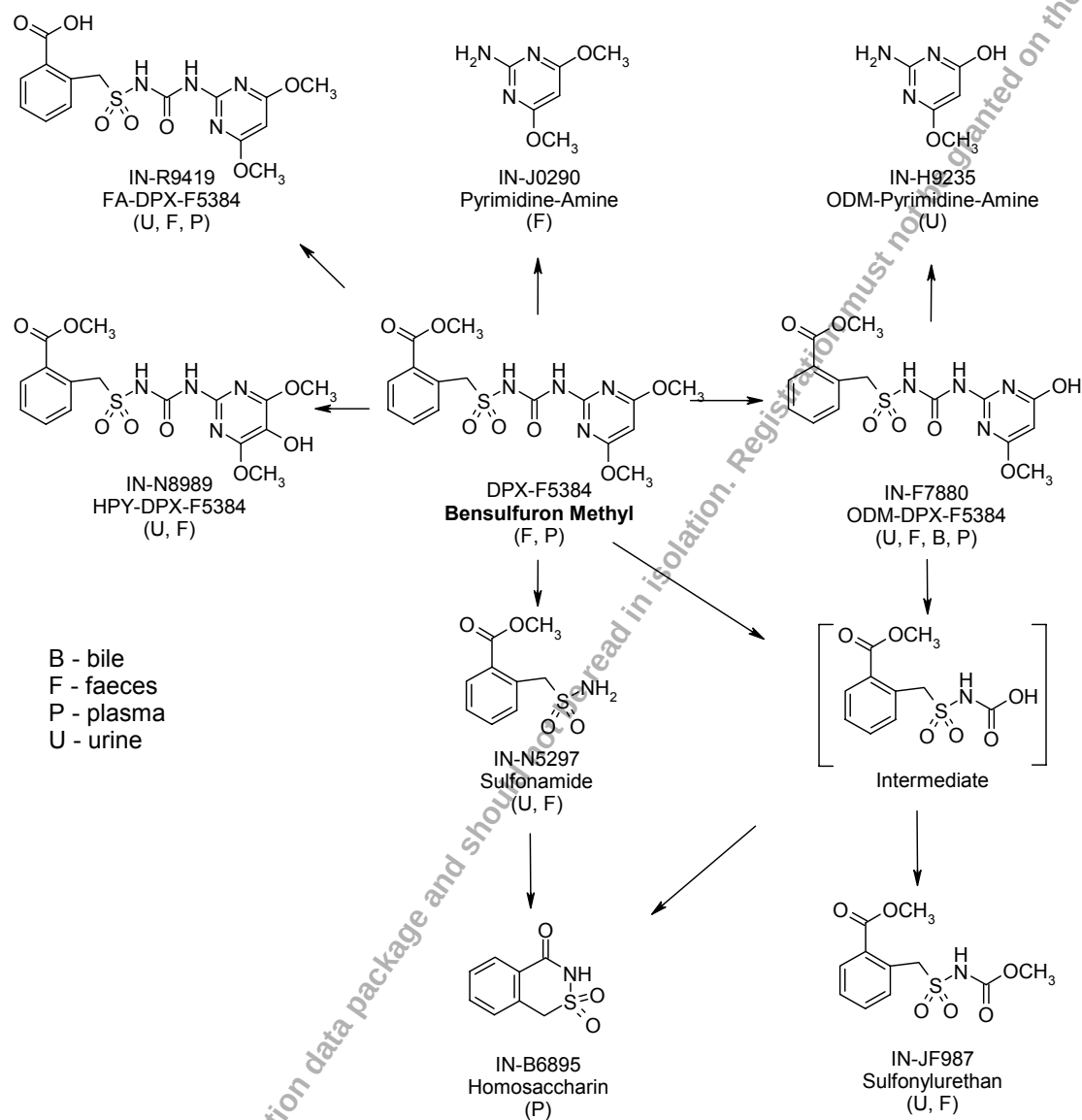
^a Identification of IN-JF987 may be an artifact of sample processing due to the use of methanol as a reagent.

^b Not reported

^c Comprised of multiple unidentified components designated as "other", "remainder", and/or resolved but uncharacterised chromatographic regions.

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

Toxicology and metabolism

Figure B.6. 1: Proposed metabolic pathway for bensulfuron methyl in the rat**B.6.1.2 Metabolism study - goat**

A metabolism study on goat was performed and is summarised in the Bensulfuron methyl EU Dossier, Annex IIA, Document M-II, Section 4, DuPont-6184 EU, Point 6.2.

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

Toxicology and metabolism

B.6.1.3 Summary of metabolism study

Table B.6. 8: Summary of metabolism studies in rats

Rate and extent of absorption:	Absorption substantially complete by 24 h after dose administration based on rise and decline of radioactivity in plasma. Peak plasma concentrations occurred at 1-4 h after oral gavage with a single low dose (20 mg/kg bw); 4 to 12 h after high dose (1000 mg/kg bw). Estimated percent dose absorbed by 24 h after administering 20 mg/kg bw was >83 to >90% as sum of dose recovered in bile, urine, cage wash, and tissues for females and males, respectively. AMR 964-87
Distribution:	The elimination half-lives describing the decline of plasma radioactivity ranged from 5.3 to 9.1 h. Less than 0.3% of the dose remained in all tissues and carcass by 96h after dose administration. The majority of the absorbed dose was excreted in bile and urine. AMR 964-87
Potential for accumulation:	Tissue-to-plasma ratios for total radioactivity were <1 for low dose and 2-5 for high dose. Residual radioactivity in plasma was undetectable by 72 h after dose administration indicating minimal potential for bio-accumulation. AMR-964-87
Rate and extent of excretion:	Excretion in urine and faeces essentially complete by 48 to 72 h after administration, independent of sex, dose level, radiolabel, or single or multiple dosing. After single or multiple low dose, the percent excreted was 44-58% of dose in urine and 27-42% in faeces. After the high dose with pyrimidine or phenyl labels, excretion in urine was 22-30% and faeces 57-66% of the dose. Biliary excretion through 24h after dose administration was 29 and 16% in male and female rats, respectively. AMR-964-87
Metabolism in animals	Bensulfuron methyl undergoes extensive metabolism in rats primarily by <i>O</i> -demethylation and hydroxylation of the pyrimidine ring as well as sulfonamide cleavage. No major changes observed between pyrimidine or phenyl labels, sexes, low and high dose groups, or single vs multiple dosing. AMR-964-87
Toxicologically significant compounds (animals, plants and environment)	Parent compound

B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

Acute toxicity studies were conducted with bensulfuron methyl technical. Summaries of these studies with the technical material to be registered are presented below.

B.6.2.1 Acute toxicity - oral

4) Median lethal dose (LD_{50}) of IN-F-5384-52 in rats - EPA proposed guidelines. Redgate, D., 1984.

Cross reference IIA, 5.2.1./01

Guidelines:

EPA Proposed Guidelines for Pesticide Registration (Federal Register Vol 43, p. 37336, August 22, 1978 Deviations: Clinical signs were not observed on weekends. This was not considered to significantly impact the determination of an LD_{50} .

GLP:

Not specified

The study is *acceptable*

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

Toxicology and metabolism

Materials and methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl in corn oil (300 mg/mL), was administered by single-dose oral gavage to fasted male and female Crl:CD® rats (5/sex) at a dose of 5000 mg/kg. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing. Rats were examined for gross pathological changes at the end of the 14-day observation period.

Findings:

No mortalities were observed (Table B.6.9). No clinical signs of toxicity were observed in male rats. Test substance-related, slight sporadic weight loss occurred in female rats. No test substance-related gross lesions were observed at necropsy.

Table B.6.9: Acute oral toxicity of bensulfuron methyl

Dose (mg/kg)	Males mortality ^a	Time of death	Dose (mg/kg)	Females mortality ^a	Time of death
5000	0/5	-	5000	0/5	-

^aumber of animals which died/number of animals in dose group

Conclusion:

The oral LD₅₀ for bensulfuron methyl was greater than 5000 mg/kg of body weight for both male and female rats. In accordance with the provisions of Directive 67/548/EEC, classification for acute toxicity is not required.

B.6.2.2 Acute toxicity – percutaneous

5) *Acute skin adsorption LD₅₀ test on rabbits with technical DPX-F5384. Gargus, J.L., 1983.*

Cross reference IIA, 5.2.2./01

Guidelines:

USEPA 81-2.

Deviations: The size of the treatment site was not specified in the report. The treatment site was abraded by design. The dressing used to ensure skin contact of test substance was made of an impervious material. These deviations are not considered to impact the determination of a dermal LD₅₀.

GLP:

Not applicable

The study is acceptable

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-38

Purity: 94%

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

Toxicology and metabolism

A single dose of bensulfuron methyl was applied to the shaved, abraded skin of 5 male and 5 female New Zealand white rabbits at a dose of 2000 mg/kg. The application site was occluded for 24 hours after which the test substance was removed. The rabbits were observed for clinical signs, body weight effects, and mortality for up to 14 days following application. Rabbits were examined for gross pathological changes at the end of the 14-day observation period.

Findings:

No mortalities were observed (Table B.6.10). No clinical signs of toxicity were observed. The rabbits exhibited no dermal irritation throughout the study. There were no test substance-related body weight effects noted. No test substance-related gross lesions were observed at necropsy.

Table B.6.10: Acute dermal toxicity of bensulfuron methyl

Dose (mg/kg)	Males mortality ^a	Time of death	Dose (mg/kg)	Females mortality ^a	Time of death
2000	0/5	-	2000	0/5	-

^a Number of animals which died/number of animals in dose group

Conclusion:

The dermal LD₅₀ for bensulfuron methyl was greater than 2000 mg/kg body weight for both male and female rabbits. In accordance with the provisions of Directive 67/548/EEC, classification for acute dermal toxicity is not required.

B.6.2.3 Acute toxicity – inhalation

6) *Inhalation median lethal concentration (LC₅₀) on IN-F5384 by EPA protocol. Burgess, B.A., 1983.*

Cross reference IIA, 5.2.3./01

Guidelines:

USEPA 81-3

Deviations: The report does not specify the number of air changes per hour in the exposure chambers. The volume of the chamber occupied by animals was not described in the report. The rate of chamber airflow was not reported. Chamber temperature exceeded the suggested guideline range of 22±3°C. Animals were not observed for clinical signs on weekends. Based on the nose-only exposure design and absence of significant signs of toxicity, neither of these deviations was considered to significantly impact the determination of an inhalation LC₅₀.

GLP:

Yes

The study is *acceptable*

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-38

Purity: 94%

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

Toxicology and metabolism

7) *Inhalation median lethal concentration (LC₅₀) of INF-5384-38 by EPA protocol. Kinney, L. A., 1987.*

Cross reference IIA, 5.2.3./02

Guidelines:

USEPA 81-3

Deviations: Not applicable

GLP:

Yes

The study is *acceptable*

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-38

Purity: 94%

Bensulfuron methyl was administered as a single four-hour, nose-only exposure to CrI:CD[®] rats. Groups of 10 male and 10 female rats each were exposed to 0, 6.6, or 7.5 mg/L of the test substance. Test atmospheres were generated by suspension of bensulfuron methyl particulate in air. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing. After 14 days of observation, 3 rats/sex/group were sacrificed for gross pathological evaluation. Lungs, liver, and kidney were examined microscopically. The main exposure parameters were as follows:

Table B.6.11: Inhalation exposure atmosphere characteristics

Parameter	Value
Mean concentration(s) ^a	6.6 mg/L
	7.5 mg/L
Gravimetric concentration range	2.4 - 10 ± 3.3 mg/L
	4.0 - 11 ± 2.4 mg/L
Particle size MMAD ^b	3.8 – 4.8 µm
Particles <3 µm (% w/w)	11 - 16%
Particles <10 µm (% w/w)	34 - 39%

^a Mean(s) were gravimetrically determined from chamber samples.

^b MMAD = mass median aerodynamic diameter of the respirable portion of the test atmosphere (measured only for the 7.5 mg/L concentration).

Findings:

No mortalities were observed (Table B.6.12). There were no clinical signs of toxicity observed in male rats. Female rats (control and test) exhibited sporadic lung noise. Females exposed to 7.5 mg/L had clear ocular discharge for 1-2 days post exposure and brown discoloured fur for 2-14 days post exposure. There was slight weight loss noted 1 day post exposure followed by normal weight gain. No test substance-related gross or microscopic lesions were observed.

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

Toxicology and metabolism

Table B.6.12: Acute inhalation toxicity of bensulfuron methyl

Concentration (mg/L)	Males mortality ^a	Time of death	Concentration (mg/L)	Females mortality ^a	Time of death
0	0/10	-	0	0/10	-
6.6	0/10	-	6.6	0/10	-
7.5	0/10	-	7.5	0/10	-

^a number of animals which died/number of animals in dose group

Conclusion:

The inhalation LC₅₀ for bensulfuron methyl in rats was greater than 5.0 mg/L for male and female rats, respectively. In accordance with the provisions of Directive 67/548/EEC, classification for acute inhalation toxicity is not required.

B.6.2.4 Acute toxicity - skin irritation

8) *Skin irritation test on rabbits for EPA pesticide registration. Gargus, J.L., Cline, E.J., 1984.*

Cross reference IIA, 5.2.4./01

Guidelines:

Proposed Guidelines for Pesticide Registration, Title 40 CFR 163, 81-5, August 22, 1978

Deviations: Four separate test sites/animal were used, of which 2 sites were abraded. The first observation occurred 24 hours after treatment instead of 1 hour after treatment. **The observation at 72 hours was not performed.** None of these are judged to significantly impact a determination of the irritation potential for bensulfuron methyl on intact skin.

GLP:

No

*The study is **acceptable***

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl (0.5 g) was applied to 2 separate sites on the shaved intact skin, and was applied to 2 shaved, abraded skin sites, on each of 6 male New Zealand White rabbits. The test substance, moistened with water, was applied to a 6 cm² area of skin. The application area was covered with a 1-inch square gauze square, which was held in place with non-irritating tape and covered with rubber sheeting for a semi-occlusive dressing. The rabbits were exposed to the test substance for 24 hours after which the test substance was removed. Test sites were evaluated by Draize criteria (1959) for signs of dermal irritation 24 and 48 hours after test substance application.

Findings:

No oedema or erythema was observed (Table B.6.13). There were no test substance-related body weight effects or clinical signs noted.

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

Table B.6.13: Individual and mean dermal irritation scores according to Draize (1959)

Animal no.	Erythema						Oedema					
	E35209	E35210	E35211	E35212	E35213	E35214	E35209	E35210	E35211	E35212	E35213	E35214
24h (intact skin)	0	0	0	0	0	0	0	0	0	0	0	0
48h (intact skin)	0	0	0	0	0	0	0	0	0	0	0	0
24h (abraded skin)	0	0	0	0	0	0	0	0	0	0	0	0
48h (abraded skin)	0	0	0	0	0	0	0	0	0	0	0	0

Conclusion:

Based on the lack of skin irritation observed at 48 hours, and according to the criteria specified in Directive 67/548/EEC, bensulfuron methyl does not classify as a skin irritant.

B.6.2.5 Acute toxicity - eye irritation

9) *Eye irritation test in rabbits - EPA pesticide registration. Gargas, J.L., Cline, E.J., 1984.*

Cross reference IIA, 5.2.5./01

Guidelines:

Environmental Protection Agency's Proposed Guidelines for Pesticide Registration, Title 40 CFR 163 81-4, August 22, 1978.

Deviations: Eyes of 3 of the 9 rabbits were washed approximately 1 minute after treatment. The eyes were not examined at 1 hour post-treatment. These deviations were minor and did not impact the determination of the irritation potential in unwashed eyes.

GLP:

No

*The study is **acceptable***

Materials and Methods:

Test substance: IN-F5384-52 technical

Batch: F5384-52

Purity: 95.9%

A single dose of 29 mg of bensulfuron methyl (equivalent to 0.1 mL) was administered into the lower conjunctival sac of the left eye of 9 female New Zealand white rabbits. The eyes of 6 rabbits were not rinsed after introduction of the test substance. Approximately 10 seconds after administration of the test substance, the treated eyes of the remaining 3 rabbits were rinsed for approximately 60 seconds with room temperature water. The conjunctiva, iris, and cornea of each treated eye were evaluated for evidence of irritation approximately 24, 48, and 72 hours following administration of the test substance. The treated eye of each rabbit was examined using fluorescein dye at 24 hours.

Findings:

The test substance produced no ocular irritation in any of the treated eyes (washed or unwashed) at any observation interval. There were no test substance-related body weight effects or clinical signs noted.

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

Toxicology and metabolism

Conclusion:

Based on the lack of eye irritation observed at 24 to 72 hours, and according to the criteria specified in Directive 67/548/EEC, bensulfuron methyl does not classify as an eye irritant.

B.6.2.6 Acute toxicity - skin sensitisation

10) *Closed-patch repeated insult dermal sensitization study (maximization method) with DPX-F5384-122 in guinea pigs. Arondi, S., 1991.*

Cross reference IIA, 5.2.6./01

Guidelines:

USEPA 81-6; OECD 406; MAFF Japan, 59 NohSan No. 4200

Deviations: None

GLP:

Yes

*The study is **acceptable***

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-122

Purity: 96.1%

The dermal sensitisation potential of bensulfuron methyl was evaluated by the Magnusson-Kligman Maximisation method in male and female Hartley albino guinea pigs. Based on a range finding study, twenty animals were intradermally induced on Day 1 with 0.1 mL of a 5% w/v suspension of bensulfuron methyl with or without Freund's complete adjuvant. Six days after intradermal induction all test and vehicle control sites were re-clipped and pre-treated with 10% sodium lauryl sulfate in petrolatum to prepare the skin for topical induction. On test day 7, animals were topically induced with 0.3 mL of the test substance suspension (25% w/v) applied to filter paper, which was then applied to the injection site. After approximately 2 weeks, animals were challenged on test day 22 with 0.2 mL of the test substance suspension and 0.2 mL of the vehicle control (petrolatum) on two separate sites. Approximately 24 and 48 hours after the challenge phase, the test sites were evaluated for signs of elicited sensitisation. Very faint redness (usually nonconfluent, score of 0.5) was not considered a positive dermal reaction. Scores of 1 (faint redness, usually confluent) or greater were required to be indicative of sensitisation. A contemporaneous positive control was treated similarly with 0.1% DNCB or petrolatum on two separate test sites.

Findings:

Results of range finding study: The intradermal irritation potential of the test substance was evaluated by exposing 4 naive animals to 0.1 mL aliquots of 0.5, 1.5, 3.0, and 5.0% (w/v) suspensions of the test substance in 0.9% saline. The injection sites were assessed after 24 and 48 hours. Slight patchy mild redness was observed in one animal at the 1.5, 3.0, and 5.0% sites and in another animal at the 5.0% site. The topical range-finding study was conducted with application of filter papers containing the test substance to intact, clipped skin at concentrations of 1.0, 5.0, 10, and 25%. No signs of irritation were observed at any dose tested. Based on these results, 5.0% was selected for intradermal induction in the main study, and 25% was selected for topical induction.

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

Toxicology and metabolism

Results of main study: During the challenge phase, slightly patchy mild redness was observed in three animals 24 hours after removal of the test substance (25% concentration), Table B.6.14. Slightly patchy mild redness was also observed at the 24 hour time point in 2 guinea pigs which had been exposed to the vehicle during the induction phase and the to 25% concentration of the test substance during the challenge phase. Since the application of the test substance during the challenge phase was the first time the vehicle control animals had been exposed to the test material, the slightly patchy mild redness observed at 24 hours in both test and vehicle groups was indicative of irritation, not sensitisation. Intense redness and swelling to severe redness and swelling were observed in the positive control animals.

Table B.6.14: Maximisation test dermal response 24 and 48 hours after challenge

Hours	Test substance group				Positive control group			
	25%		Petrolatum		0.1% DNCB		Petrolatum	
	24	48	24	48	24	48	24	48
	3/20 ^a	2/20	0/20	0/20	6/6	6/6	0/6	0/6

Hours	Vehicle control group			
	25%		Petrolatum	
	24	48	24	48
	2/20 ^a	0/20	0/20	0/20

^a Number of animals with positive dermal response/number of animals in dose group

Conclusion:

Bensulfuron methyl does not possess skin sensitising potential under the conditions of the Maximisation test.

B.6.2.7 Summary of acute toxicity

Table B.6.15: Summary of acute toxicity studies with bensulfuron methyl

Parameter	Species	Result	Reference
Acute oral LD ₅₀	Rat	>5000 mg/kg	Redgate, 1984
Acute dermal LD ₅₀	Rabbit	>2000 mg/kg	Gargus, 1983
Acute inhalation LC ₅₀ (4h)	Rat	>5.0 mg/L	Burgess, 1983
Acute skin irritation	Rabbit	Non irritant	Gargus and Cline, 1984
Acute eye irritation	Rabbit	Non irritant	Gargus and Cline, 1984
Skin Sensitisation Maximisation test	Guinea Pig	Non sensitiser	Armondi, 1991

Bensulfuron methyl has no significant acute toxicity *via* the oral, dermal and inhalation routes of exposure. Bensulfuron methyl is not an eye or skin irritant and does not cause skin sensitisation.

B.6.3 Short-term toxicity (Annex IIA 5.3)

In addition to the 90-day feeding study in rats, several other short-term toxicity studies were conducted with bensulfuron methyl, including 90-day feeding studies in mice and dogs, and a 1-year feeding study in dogs.

B.6.3.1 Oral 14- and 28-day studies

It is not mandatory to conduct either 14-day or 28-day oral studies, and no studies with bensulfuron methyl were conducted.

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

Toxicology and metabolism

B.6.3.2 Oral 90-day and 1-year studies

Subchronic (90-day) feeding studies were conducted with bensulfuron methyl in rats, mice, and dogs. A 1-year feeding study was conducted in dogs.

B.6.3.3 Oral 90-day study – rats

11) *Ninety-day feeding and one-generation reproduction study in rats with benzoic acid, 2-((((4,6-dimethoxy-2-pyrimidinyl)amino)-carbonyl)amino)sulfonylmethyl)-methyl ester, (INF-5384), O'Neal, F.O., 1986.*

Cross Reference IIA, 5.3.2./04

Guidelines:

Not specified

Deviations: An ophthalmological exam was not conducted, however, eyes were collected and evaluated microscopically. The following clinical pathology parameters were not measured: Serum phosphorus, chloride, bilirubin, and total serum protein. Adrenal glands were not weighed. The following organs were not evaluated microscopically: Lymph node, peripheral nerve, sternum with bone marrow, and rectum. Since many of the clinical observations, clinical chemistry, gross and histopathological parameters provide multiple indicators of organ dysfunction, these deviations were not considered to impact the ability of the study to detect adverse effects.

GLP:

No

The study is *acceptable*

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-15, F5384-17, F5384-18

Purity: 95.7%, 98.5%, and 98.4%, respectively.

12) *Supplementary tables required for registration in Japan. Stula, E.F., 1986.*

Cross reference IIA, 5.3.2./07

Guidelines:

Not specified

Deviations: Not applicable

GLP:

No

The study is *acceptable*

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-15, F5384-17, F5384-18

Purity: 95.7%, 98.5%, and 98.4%, respectively.

WARNING: This document forms part of an EC evaluation data package and should not be used as a basis for registration. 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

Toxicology and metabolism

In a 90-day feeding study, bensulfuron methyl was administered to male and female CD[®] rats (16 rats/sex/concentration) at concentrations of 0, 100, 1500, or 7500 ppm. The mean daily intakes for male rats were 0, 6, 93, and 475 mg/kg bw/day. The mean daily intakes for female rats were 0, 7, 111, and 567 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, and organ weights. At the end of the 90-day feeding period, 10 rats/sex/group were sacrificed and necropsied. Tissues were collected and evaluated microscopically, including eyes. The remaining 6 rats/sex/group were mated and allowed to deliver litters.

Findings:

General observations: The observations for rats fed test diets were unremarkable and occurred with frequencies comparable to controls. Test substance-related mortality did not occur during the course of this study. There were no test substance-related effects on body weights, body weight gains, food consumption or food efficiency.

Haematology, clinical chemistry, and urinalysis: Male rats fed 7500 ppm had statistically significant decreases in erythrocyte counts, haemoglobin content haematocrit, and alanine and aspartate aminotransferase activities at the 3-month evaluation (Table B.6.16). In addition, 7500 ppm males had increased serum cholesterol concentrations at the 1, 2, and 3 month evaluations. Female rats fed 1500 or 7500 ppm had statistically significant decreases in platelet counts at the 2- and 3-month evaluations. However, these decreases were not considered to be adverse because they were within the range of biological variation. There were no remarkable observations for urinalysis parameters at any dose level.

Table B.6.16: Summary of haematology and clinical chemical values for male and female rats administered bensulfuron for 90-days

	0 ppm	100 ppm	1500 ppm	7500 ppm
Males:				
RBC (MM/mm)	8.1	7.7	8.1	7.3 ^a
Hb (g/dL)	17.0	15.9	16.7	15.8 ^a
Hct (%)	42	40	41	37 ^a
ALT (IU)	30	24	26	26 ^a
AST (IU)	98	83	84	77 ^a
Cholesterol (mg %)	71	91	74	98 ^a
Females:				
RBC (MM/mm)	7.5	7.6	7.8	7.3
Hb (g/dL)	16.4	16.4	16.5	16.0
Hct (%)	41	41	43	40
ALT (IU)	27	27	24	26
AST (IU)	93	91	86	77
Cholesterol (mg %)	75	84	67	82

^a Statistically different (p < 0.05) from control by Dunnett's test

Gross pathology, organ weights, and histopathology: No test substance-related gross lesions were observed at necropsy. Changes in liver weight and/or morphology occurred in 7500 ppm males and females. Absolute and relative liver weights (% body weight) were increased in 7500 ppm males and females (Table B.6.17). Absolute liver weight was also increased in 100, 1500 and 7500 ppm females. Absolute heart weight was increased in 7500 ppm females, and relative brain weight was decreased in 100, 1500, and 7500 ppm females. Relative kidney weight was also decreased in 1500 and 7500 ppm groups. Changes in absolute heart weight, relative brain weight, and relative

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kidney weight were secondary to the greater body weights of 100, 1500, and 7500 ppm females compared to control. Histologically, livers from 7500 ppm males showed markedly decreased staining of cells within the centrilobular region. However, in the absence of clinical chemical or histopathological data that would indicate otherwise, the decreased staining, in male rats and the increased absolute and relative liver weights in 7500 males and females were not considered to be adverse, and may be secondary to an adaptive response of the liver to treatment with the test substance. Although an ophthalmoscopic examination was not conducted prior to sacrifice, eyes were collected and evaluated microscopically. No test substance-related gross or microscopic lesions in the eye were detected.

Table B.6.17: Organ and body weights of rats administered bensulfuron for 90-days

	0 ppm	100 ppm	1500 ppm	7500 ppm
Males:				
Body weight (g)	516.1	523.6	522.8	511.5
Absolute liver weight (g)	19.179	18.965	18.717	21.863 ^b
Relative ^a liver weight	3.61	3.82	3.55	4.02 ^b
Females:				
Body weight (g)	240.9	268.5 ^b	263.7 ^c	269.1 ^b
Absolute liver weight (g)	8.290	9.644 ^b	9.473 ^b	10.418 ^b
Relative ^a liver weight	3.444	3.592	3.596	3.875 ^b
Absolute kidney weight (g)	2.055	2.091	1.945	2.078
Relative ^a kidney weight	0.852	0.782	0.739 ^b	0.773 ^c
Absolute brain weight (g)	1.974	1.944	1.939	1.921
Relative ^a brain weight	0.811	0.730 ^b	0.740 ^c	0.718 ^b
Absolute heart weight (g)	0.868	0.945	0.941	0.981 ^b
Relative heart ^a weight	0.362	0.353	0.358	0.366

^a Percent of body weight

^b Statistically different (p < 0.05) from control by LSD and Dunnett's test

^c Statistically different (p < 0.05) from control by LSD

Reproductive parameters: The fertility rate and average number of offspring per litter for the control-group rats were uncharacteristically low. However, the fertility of the test groups was within the expected range. In addition, the gestation index, percent of pups born alive, percent of pups that survived 4 days (viability index), percent of pups that survived to 21 days (lactation index), litter survival, and body weight of weanlings were comparable for all treated and control groups. The clinical appearance of the pups was also comparable among all groups with the exception of one litter in the 7500 ppm group. Two of 6 pups in the affected litter had poor fur development. In addition, the litter had poor survival to lactation day 21, and low pup weights. It cannot be determined from this study whether the effects on one litter in the 7500 ppm group were test substance-related. This was determined in the definitive multigeneration rat reproduction study reported under Point 5.6.1 of this document.

Conclusion:

The systemic NOAEL in the 90-day feeding study was 1500 ppm (93 mg/kg/day) for male rats based on the haematology effects observed at 7500 ppm. The NOAEL in female rats was 7500 ppm (567 mg/kg/day) based on no adverse effects at any dietary concentration.

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Toxicology and metabolism

B.6.3.4 Oral 13-week study – mice

13) *DPX-F5384: 13-week oral subacute toxicity study in mice. Hirano, M., 1984.*

Cross reference IIA, 5.3.2./03

Guidelines:

Not specified

Deviations: An ophthalmoscopic exam was not conducted, however eyes were collected at necropsy, and were examined microscopically. The following clinical pathology parameters were not measured: serum phosphorus, chloride, sodium, blood creatine, and total bilirubin. Because of low blood volumes obtainable from mice, evaluations were limited to haematology. Because of the mode of action and known target organs, these parameters did not significantly impact data interpretation.

GLP:

No

*The study is **acceptable***

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-44

Purity: >95%

In a 90-day feeding study, bensulfuron methyl was administered to male and female ICR-JCL mice (20/sex/concentration) at concentrations of 0, 300, 1000, 3000, or 10,000 ppm. The mean daily intakes for male mice were 0, 38.9, 132, 387, and 1272 mg/kg bw/day. The mean daily intakes for female mice were 0, 39.2, 133, 407, and 1401 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food and water consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology.

Findings:

General observations: There were no test substance-related clinical signs observed during the course of the study. One 1000 ppm female was sacrificed *in extremis* on test day 55 due to lymphosarcomatosis. There were no test substance-related effects on body weights, body weight gains, food or water consumption, and food efficiency.

Haematology, clinical chemistry and urinalysis: Significant increase in mean corpuscular haemoglobin concentration (MCHC) was observed in 10,000 ppm females; however, since other haematology parameters were not affected, this was not considered to be adverse. There were no test substance-related effects on urinalysis parameters in either sex at any dietary concentration.

Gross pathology, organ weights, histopathology: Male and female mice in the 10,000 ppm group had a higher incidence of liver enlargement and/or cloudy colour of the liver which was also observed in a few 3000 ppm males. No other test substance-related gross findings were observed. Significant increases in absolute and relative liver weights were observed in 10,000 ppm males and females, 3000 ppm males, 1000 ppm males, and 300 ppm females (Table B.6.18). Significant increases in only relative liver weights were observed in 1000 and 3000 ppm females. The increases in liver weight relative to body weight in 300, 1000, and 3000 ppm females were not dose-related and

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liver weight relative to brain weight was not affected in these groups. Therefore, the statistically significant increases in absolute and relative liver weight in 300, 1000, and 3000 ppm females were not considered to be test substance-related. An increase in relative heart weights was also noted in 3000 ppm females, but the change was not dose related.

Test substance-related, statistically significant increases in the incidence of centrilobular swelling of hepatocytes was observed in the livers of 10,000 ppm males and females and in 3000 ppm males (Table B.6.19). Although test substance-related increases in liver weight parameters and centrilobular swelling of hepatocytes was observed at 3000 and 10,000 ppm, these effects are not considered to be adverse since they are an adaptive response to metabolism of a xenobiotic.

Although an ophthalmoscopic evaluation was not conducted prior to sacrifice, eyes were collected and examined microscopically. No test substance-related gross or microscopic lesions in the eye were detected.

Table B.6.18: 90-Day feeding study in mice: Organ weights

Bensulfuron methyl (ppm)	0 ppm	300 ppm	1000 ppm	3000 ppm	10,000 ppm
Males:					
Absolute liver weight (g)	2.32	2.40	2.53 ^b	2.55 ^c	2.79 ^c
Relative ^a liver weight	5.31	5.45	5.86 ^c	5.80 ^c	6.48 ^c
Liver to brain weight	4.63	4.77	5.06 ^d	5.16 ^e	5.65 ^e
Absolute heart weight (mg)	211	206	206	202	196
Relative ^a heart weight	0.49	0.47	0.48	0.46	0.45
Females:					
Absolute liver weight (g)	1.74	1.98 ^b	1.89	1.95	2.27 ^c
Relative ^a liver weight	4.41	5.05 ^c	5.01 ^c	5.27 ^c	5.91 ^c
Liver to brain weight	3.43	3.84	3.70	3.80	4.42 ^e
Absolute heart weight (mg)	147	153	149	152	152
Relative ^a heart weight	0.37	0.39	0.40	0.41 ^b	0.40

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by the Fisher's Exact test criteria, p = 5%.

^c Significantly different from control by the Fisher's Exact test criteria, p = 1%.

^d Significantly different from control by Dunnett's multiple comparison test criteria p < 0.05.

^e Significantly different from control by Dunnett's multiple comparison test criteria p < 0.01.

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Toxicology and metabolism

Table B.6.19: 90-Day feeding study in mice: Incidences of microscopic effects

Bensulfuron methyl (ppm):	0	300	1000	3000	10,000
Number of mice/group:	M	20	20	20	20
	F	20	20	19	20
Males:					
Liver					
Centrilobular swelling of hepatocytes	0 ^a	1	4	8 ^b	19 ^c
Hepatocellular necrosis	1	0	2	0	1
Microgranulomas	0	1	0	1	1
Hematopoietic foci	0	2	0	1	0
Females:					
Liver					
Centrilobular swelling of hepatocytes	0	1	0	1	9 ^c
Fatty change of hepatocytes	1	0	1	0	0
Hepatocellular necrosis	0	0	1	1	1
Microgranulomas	4	2	2	0	2
Hematopoietic foci	1	3	0	0	0

^a Number of organs with microscopic change.^b Significantly different from control by the Fisher's Exact test criteria, $p = 1\%$.^c Significantly different from control by the Fisher's Exact test criteria, $p = 0.1\%$.Conclusion:

The NOAEL in the 90-day feeding study in mice was 10,000 ppm for males and females (1272 and 1401 mg/kg/day for males and females, respectively) based on the absence of adverse effects at dietary concentrations up to 10,000 ppm.

B.6.3.5 Oral 90-day study - dogs

14) *Three-month feeding study in dogs with INF-5384. Stadler, J.C., 1986.*

Cross reference IIA, 5.3.2./05

Guidelines:

Not specified.

Deviations: An ophthalmoscopic examination was not conducted; however, eyes were collected and evaluated histologically. Serum phosphorous was not measured during the clinical chemistry appraisal. Because of the mode of action and known target organs, these parameters did not significantly impact data interpretation.

GLP:

Yes

The study is *acceptable*

Materials and methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

In a 90-day feeding study, bensulfuron methyl was administered to male and female beagle dogs (4 dogs/sex/concentration) at concentrations of 0, 100, 1000, or 10,000 ppm. The mean daily intakes for male dogs were 0, 3.3, 32, or 341 mg/kg bw/day. The mean daily intakes for female dogs were 0, 3.5, 37, or 359 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology.

Findings:

General observations: There were no test substance-related clinical signs of toxicity observed during the course of the study. Test substance-related mortality did not occur. Body weights and body weight gains were lower than controls in the 10,000 ppm males and females. The lower body weights and body weight gains were not significantly different from control but were considered to be test substance-related. There were no test substance-related effects on food consumption at any dose level, however, test substance-related decreases in food efficiency occurred in males and females administered 10,000 ppm bensulfuron methyl (43% lower in males and 31% lower in females compared to control values).

Haematology, clinical chemistry, urinalysis: Test substance-related effects on clinical chemistry parameters were observed at the 1-, 2-, and 3-month evaluations in 10,000 ppm males and females. Statistically significant elevations in the activities of alkaline phosphatase and alanine aminotransferase were considered to be indicative of a test substance-related hepatotoxic effect (Table B.6.20). No other clinical pathology observations were determined to be biologically significant.

Table B.6.20: 90-Day feeding study in dogs: Clinical chemistry evaluation

	0 ppm			100 ppm			1000 ppm			10,000 ppm		
	1mo	2mo	3mo	1mo	2mo	3mo	1mo	2mo	3mo	1mo	2mo	3mo
Males:												
ALP (Units/L)	107	96	81	106	90	82	89	79	66	157	179 ^a	213 ^a
ALT (Units/L)	20	28	27	24	28	30	20	23	27	37 ^a	78 ^a	70 ^a
	1mo	2mo	3mo	1mo	2mo	3mo	1mo	2mo	3mo	1mo	2mo	3mo
Females:												
ALP (Units/L)	97	84	80	87	76	69	89	120	73	197 ^a	242 ^a	261 ^a
ALT (Units/L)	21	25	24	27	32	32	31	28	27	44 ^a	89 ^a	75 ^a

ALP Alkaline phosphatase

ALT Alanine aminotransferase

^aStatistically significant at $p < 0.05$ by Dunnett's criteria

Gross pathology, organ weights, histopathology: A number of gross and histopathological changes were observed in dogs fed diets that contained 10,000 ppm bensulfuron methyl. Gross observations at necropsy included dark livers in 10,000 ppm males and females and calculi in the gallbladders of 2 of 4 males. Microscopic lesions included bile stasis and centrilobular hepatocellular swelling in 10,000 ppm males and females, and hepatocyte necrosis in 10,000 ppm females (Table B.6.22). In addition, 3 of 4 males in the 10,000 ppm group had microscopic evidence of gallbladder calculi. Absolute and relative liver weights were increased in 10,000 ppm males and females. There were no biologically significant pathologic changes observed in any dogs fed 1000 ppm or below (Table B.6.21). Although an ophthalmoscopic evaluation was not conducted prior to sacrifice, eyes were collected and evaluated microscopically. No test substance-related gross or microscopic lesions in the eyes were detected.

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Table B.6.21: 90-Day feeding study in dogs: Organ weights

	0 ppm	100 ppm	1000 ppm	10,000 ppm
Males:				
Absolute liver weight (g)	366.4	384.1	373.5	443.5 ^b
Relative ^a liver weight	3.18	3.26	3.24	4.24 ^b
Females:				
Absolute liver weight (g)	341.5	337.7	376.8	424.8 ^b
Relative ^a liver weight	3.24	3.22	3.68	4.34 ^b

^a Relative weight is defined as the organ to body weight ratio.^b Significantly different from control by the LSD and Dunnett's criteria, $p < 0.05$.**Table B.6.22: 90-Day feeding study in dogs: Incidences of microscopic effects**

Bensulfuron methyl (ppm):	0	100	1000	10,000
Number of dogs/group:	4	4	4	4
Males:				
Liver				
Bile stasis	0 ^a	0	0	4
Hepatitis, focal/multifocal	4	4	4	4
Hepatocellular swelling, centrilobular	0	0	0	4
Mixed inflammatory cell infiltrate, centrilobular	3	2	1	2
Mixed inflammatory cell infiltrate, periportal	1	0	1	0
Gallbladder				
Calculus	0	0	0	3
Lymphocytic infiltration/hyperplasia	4	4	1	2
Females:				
Liver				
Bile stasis	0	0	0	4
Granuloma	1	0	0	0
Hepatitis, focal/multifocal	4	4	4	4
Hepatocellular swelling, centrilobular	0	0	0	4
Individual hepatocyte necrosis	0	0	0	4
Mixed inflammatory cell infiltrate, centrilobular	2	3	2	3
Mixed inflammatory cell infiltrate, periportal	2	2	1	1
Gallbladder				
Lymphocytic infiltration/hyperplasia	0	2	3	1

^a Number of organs with microscopic change.**Conclusion:**

The NOAEL in the 90-day feeding study in dogs was 1000 ppm for males and females (32 and 37 mg/kg/day, respectively). This NOAEL was based on clinical chemistry changes, and gross and microscopic tissue findings in males and females at 10,000 ppm.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

B.6.3.6 Oral 1-year study - dogs

15) *One-year feeding study in dogs with DPX-F5384 (5 Volumes). Daly, I.W., 1986.*

Cross reference IIA, 5.3.2./02

Guidelines:

USEPA 83-1

Deviations: Urine volume was not recorded. An ophthalmological evaluation was not conducted, however, 7 additional serial sections of each eye were collected from dogs in the control and high dose group (7500 ppm), and were evaluated microscopically. Evaluation of these serial sections further increased the sensitivity of the microscopic examinations and ensured that any significant effects would be observed.

GLP:

Yes

*The study is **acceptable***

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

16) *A one-year feeding study in dogs with H14793 (Addendum No. 1). Bolte, H.F., 1986.*

Cross reference IIA, 5.3.2./01

Guidelines:

Not applicable

Deviations: Not applicable

GLP:

No

*The study is **acceptable***

Material and methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

17) *Chronic toxicity study with IN F5384 one-year feeding study in dogs supplemental report. Stula, E.F., 1988.*

Cross reference IIA, 5.3.2./06

Guidelines:

Not applicable

Deviations: Not applicable

WARNING: This document forms part of a 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

Toxicology and metabolism

GLP:

Yes

*The study is **acceptable***Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

In a 1-year feeding study, bensulfuron methyl was administered to male and female beagle dogs (5 dogs/sex/concentration) at concentrations of 0, 50, 750, and 7500 ppm. The mean daily intakes for male dogs were 0, 1.4, 21.4, and 237.3 mg/kg bw/day. The mean daily intakes for female dogs were 0, 1.4, 19.9, and 222.6 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology.

Findings:

General observations: There were no test substance-related clinical signs of toxicity noted. Test substance-related mortality did not occur during the course of this study. There were no test substance-related effects on body weights or body weight gains. Food consumption in 7500 ppm males and females was 8 and 12% greater than the control values, respectively. Food efficiency was similar to control values. The report author concluded that the increased food consumption was compound-related. However, since no effects on body weight or food efficiency were observed, it was not considered to be an adverse effect.

Haematology, clinical chemistry, urinalysis: There were no test substance-related effects on haematology or urinalysis parameters during the course of the study. Mean alanine aminotransferase levels were increased compared to controls in the 7500 ppm females at the 6, 9, and 12 month bleeding intervals and in the 7500 ppm males at all bleeding intervals. Additionally, mean alkaline phosphatase levels were increased in 7500 ppm males and females at all bleeding intervals. Representative data from the 12-month interval is summarised in the following (Table B.6.23):

Table B.6.23: One-year feeding study in dogs: 12-Month clinical chemistry evaluation

	0 ppm	50 ppm	750 ppm	7500 ppm
Males:				
ALT (Units/L)	21	23	27	60 ^a
ALP (Units/L)	34	33	39	164 ^a
Females:				
ALT (Units/L)	21	23	24	43 ^a
ALP (Units/L)	52	35	53	160

^a Significantly different from control by the Dunnett's criteria, $p < 0.05$.

ALT Alanine aminotransferase

ALP Alkaline phosphatase

Gross pathology, organ weights, histopathology: Males in the 7500 ppm group exhibited a 27% increase in mean liver weights and mean liver to body weight ratios compared to controls (Table B.6.24). Slight increases were also noted in the mean liver weights (5%) and liver to body weight ratios (11%) of the 7500 ppm females. With the exception of the presence of a brown pigmented material in the bile canaliculi of the 7500 ppm males and females, there were no test substance-related gross or histopathologic findings.

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Toxicology and metabolism

Table B.6.24: One-year feeding study in dogs: Organ weights

	0 ppm	50 ppm	750 ppm	7500 ppm
Males:				
Absolute liver weight (g)	295.4	315.9	294.2	375.5 ^b
Relative ^a liver weight	2.71	2.73	2.57	3.45 ^b
Females:				
Absolute liver weight (g)	286.9	259.5	273.5	301.0
Relative ^a liver weight	2.84	2.68	3.02	3.16

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by the Dunnett's criteria, $p < 0.05$.

Conclusion:

The NOAEL in the 1-year feeding study in dogs was 750 ppm (21.4 and 19.9 mg/kg/day, respectively) for males and females. This NOAEL was based on clinical chemistry and organ weight effects at 7500 ppm.

B.6.3.7 Other routes

Short-term studies conducted by the dermal or inhalation routes of exposure were not conducted with bensulfuron methyl.

B.6.3.8 Summary of short-term toxicity

Results of short-term toxicity studies with bensulfuron methyl are summarised in Table B.6.25.

Table B.6.25: Summary of short-term toxicity studies

Type of study	Species	Dose range tested	NOAEL		Reference
			ppm	mg/kg/d	
Oral (feeding), 90-day	rat	0, 100, 1500, 7500 ppm	1500 M 7500 F	m: 93 f: 567	O'Neal, 1986
Oral (feeding), 90-day	mouse	0, 300, 1000, 3000, 10,000 ppm	10,000	m: 1272 f: 1401	Hirano, 1984
Oral (feeding), 90-day	dog	0, 100, 1000, 10,000 ppm	1000	m: 32 f: 37	Stadler, 1986
Oral (feeding), 1-year	dog	0, 50, 750, 7500 ppm	750	m: 21.4 f: 19.9	Daly, 1986

Based on the result of short-term (90-day, and 1-year) studies in the rat, mouse, and dog, the effects of bensulfuron methyl are characterised primarily by changes in haematology parameters (rats and mice), changes in serum enzyme activity, and liver enlargement. In rats, the liver enlargement was not associated with microscopic changes. In mice, the liver enlargement was associated with centrilobular swelling of hepatocytes, which was not considered to be adverse since it is a common pharmacological response to metabolism of a xenobiotic. In dogs, the liver enlargement was accompanied by microscopic changes including bile stasis, centrilobular hepatocellular swelling, and individual hepatocyte necrosis at high concentrations (10,000 ppm). However, at lower concentrations, of 7500 ppm for one-year, liver enlargement occurred in the absence of adverse histological changes. The NOAELS in rats and dogs were comparable, however, mice were the least sensitive of the species.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

B.6.4 Genotoxicity testing (Annex IIA 5.4)

A battery of *in vitro* and *in vivo* tests was conducted to determine the genotoxic potential of bensulfuron methyl.

B.6.4.1 *In vitro* studies

B.6.4.1.1 *In-vitro* gene mutation - bacteria

18) *Mutagenicity evaluation of INF-5384-7 in Salmonella typhimurium (Revision No. 2): Arce, G.T., 1987.*

Cross reference IIA, 5.4.1./01

Guidelines:

USEPA 84-2

GLP:

Yes

The study is acceptable

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-7

Purity: 95%

Bensulfuron methyl was evaluated for mutagenicity in *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 with and without an exogenous metabolic activation system (Aroclor® 1254-induced rat liver S9 (10% w/v), 1.6 mg/mL protein). Nominal concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 25 µg/plate were evaluated in 2 trials using standard plate incorporation methods. The highest dose level was set based on cytotoxicity and preliminary mutagenicity testing in the presence and absence of an activation system, as measured in strain TA1535. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). Positive indicators were 2-aminoanthracene, 9-aminoacridine, N-methyl-N-nitro-N-nitrosoguanidine, and 2-nitrofluorene. A test substance was classified as positive when the probability was less than 0.01 that the numbers of revertants at one or more of the test sample concentrations studied were not greater than the number of revertants in the solvent control, and the probability was less than 0.01 that there was not a positive correlation between the number of revertants and increasing concentrations of the test samples.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation (). Plates treated with the positive indicators had significantly increased numbers of revertant colonies per plate when compared to the solvent control. Slight cytotoxicity was observed on higher test substance concentrations.

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Italy	May 2006	Bensulfuron methyl

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Table B.6.26: Summary of mean revertants/plate

Mean ^a revertants/plate without activation									
Compound	Conc. µg/plate	TA1535		TA1537		TA98		TA100	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
DMSO	0	31	29.5	13	11	22.4	21.5	136	113
Bensulfuron methyl	0.01	ne	30.5	ne	7	ne	20.5	ne	109
	0.05	ne	28	ne	10.5	ne	19.5	ne	132.5
	0.1	32	23.5	6.5	6.5	26	22.5	143	117
	0.5	25	22.5	13.5	7.5	24	20.5	107.5	124.5
	1	32.5	25	8.5	8	26	14	118.5	115
	5	24	19	6.5	4.5	15.5	19.5	110.5	97
	10	19	ne	4	ne	16.5	ne	110.5	ne
	25	10	ne	2.5	ne	12.5	ne	82.5	ne
9AAc	50	ne	ne	1131.5	852.5	ne	ne	ne	ne
2NF	25	ne	ne	ne	ne	2194	2079	ne	ne
MNNG	4	3594.5	2958.5	ne	ne	ne	ne	3582	2572.5
Mean ^a revertants/plate with activation									
DMSO	0	22.5	17	14.5	7.5	37	31	136.5	137
Bensulfuron methyl	0.01	ne	15.5	ne	15	ne	31	ne	141
	0.05	ne	16	ne	13	ne	36	ne	157.5
	0.1	8	14.5	12.5	9	39.5	29.5	135	133.5
	0.5	18	19.5	9.5	12	32	32.5	131.5	133.5
	1	23	8	14.5	7	37	26	129.5	151
	5	14.5	16.5	3.5	9.5	32.5	29	119.5	126.5
	10	15.5	ne	6	ne	33	ne	100.5	ne
	25	12	ne	3.5	ne	20.5	ne	102	ne
2AA	1	ne	ne	ne	ne	ne	ne	1403	1116
	2	509.5	255.5	765	424.5	3107.5	2013.5	ne	ne

^a Average 2 replicates per trial9AAc = 9-aminoacridine; 2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; DMSO = dimethyl sulfoxide
ne = Not evaluated

Conclusion: Bensulfuron methyl was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

B.6.4.1.2 In-vitro mammalian cytogenicity test - clastogenicity in human lymphocytes

19) In vitro evaluation of IN-F5384-122 for chromosome aberrations in human lymphocytes. Covell, D.L., 1988.

Cross reference IIA, 5.4.1./02

Guidelines

USEPA 84-2

GLP

Yes

The study is acceptable

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

Toxicology and metabolism

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-122

Purity: 97.3%

Bensulfuron methyl was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor® 1254-induced rat liver S9). Doses in the initial and independent repeat assays were 0.1, 0.5, 0.9, and 1.1 mg/mL. The highest dose level was set based on solubility of the test substance. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 110 mg/mL. Mitomycin C and cyclophosphamide were used as the positive control for the non-activated and S9 activated studies, respectively. HPBL were treated for 3 hours, and, after exposure to Colcemid®, metaphase cells were harvested about 24 hours following the initiation of treatment. For each trial with or without activation, a minimum of 100 cells (50 from each replicate), where possible, were analyzed for each test level and control. Cells were evaluated for toxicity (mitotic inhibition) then structural chromosome aberrations. Numerical chromosome aberrations were not evaluated. The test substance was considered to induce a positive response if the percentages of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent vehicle control group ($p \leq 0.05$) or if a reproducible and significant increase in the percentage of cells with aberrations occurred at a single dose level relative to concurrent negative (or vehicle) controls.

Findings:

No statistically significant increases in structural chromosome aberrations were observed in either trial at any of the concentrations evaluated (). There was no evidence of toxicity by the test compound.

Table B.6.27 Summary of chromosome aberration data

Treatment	Conc.mg/mL	Initial assay		Repeat assay	
		% of cells with structural aberrations:		% of cells with structural aberrations:	
		S9- 3 hrs	S9+ 3 hrs	S9- 3 hrs	S9+ 3 hrs
Untreated	0	1.0	4.0	2.0	2.0
DMSO (1%)	0	5.0	4.0	4.0	0.0
Bensulfuron methyl	0.1	0.0	6.0	0.0	2.0
	0.5	1.0	3.0	3.0	4.0
	0.9	1.0	1.0	2.0	1.0
	1.1	6.0	3.0	1.0	0.0
MMC	0.30 µg/mL	34.0 ^a	ne	18.0 ^b	ne
CP	0.10 µg/mL	ne	38.0 ^a	ne	32 ^a

MMC = mitomycin C; CP = cyclophosphamide; DMSO = dimethyl sulfoxide

^a Statistically significant ($p < 0.001$; Fisher's exact test)

^b Statistically significant ($p < 0.01$; Fisher's exact test)

ne = Not evaluated

Conclusion:

Bensulfuron methyl was negative for chromosome aberrations in the non-activated and S9-activated test systems in the *in vitro* mammalian cytogenetics test using human peripheral blood lymphocytes.

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

Toxicology and metabolism

B.6.4.2 *In-vitro* gene mutation - mammalian cells

20) *CHO/HGPRT assay for gene mutation. Chromey, N.C., 1984.*

Cross reference IIA, 5.4.1./03

Guidelines:

Not specified

GLP:

No

The study is acceptable

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl was tested in the CHO/HGPRT mutation assay with and without an exogenous metabolic activation system (Aroclor[®]-induced rat liver S9, 3.0 mg/mL protein). Both an initial and an independent repeat assay were conducted. Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K₁-BH₄ cells were exposed for 18-19 hours in the nonactivated test system, and for 5 hours in the S9 activated test system, at 37 ± 1°C to the test substance at concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 mM. The highest dose level was set based on solubility. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the 6-Thioguanine (TG, 2-amino-6-mercaptopurine)-resistant phenotype. The test substance was dissolved in dimethyl sulfoxide (DMSO). Ethyl methanesulfonic acid and 9,10-dimethyl-1,2-benzanthracene were used as positive controls for the non-activated and activated test systems, respectively. The assay was considered positive when the mutant frequency of one or more of the sample concentrations tested was significantly greater than that of the solvent control, and the correlation between the mutant frequency and the concentration of the test sample was significantly greater than 0. Significance was judged at the 0.01 level.

Findings:

There were no positive responses and no evidence of toxicity in any of the assays at any of the concentrations of the test substance evaluated (). A fine precipitate was observed in the treatment medium at the highest concentration tested.

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

Toxicology and metabolism

Table B.6.28. *In vitro* mammalian gene mutation: Summary of mutagenicity findings

Compound	Conc.	Mutation frequency without activation ^a		Mutation frequency with activation ^a	
	mM	Trial 1	Trial 2	Trial 1	Trial 2
DMSO	0	12.6	1.75	6.0	23.8
Bensulfuron methyl	0.5	15.8	4.15	36.35	6.3
	1.0	1.45	3.9	9.7	1.3
	2.0	8.1	0	14.85	7.65
	3.0	13.0	10.15	28.2	7.85
	4.0	24.1	0	7.2	22.15
EMS	0.5	106.0	221.75	ne	ne
DMBA	0.015	ne	ne	222.05	199.7

^a Average of 2 x 5 plates: mutants per 1 x 10⁶ surviving cells defined as:

total mutant colonies/(number of selection dishes x cloning efficiency x 2 x 10⁵ cells) x 10⁶

EMS = Ethyl methanesulfonate; DMBA = 9,10-dimethyl-1,2-benzanthracene; DMSO = dimethyl sulfoxide

ne Not evaluated

Conclusion:

Bensulfuron methyl was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.

B.6.4.3 *In-vitro* unscheduled DNA synthesis

21) *Unscheduled DNA synthesis/rat hepatocytes in-vitro*. Chromey, N.C., McCooey, K.T., 1984.

Cross reference IIA, 5.4.1./04

Guidelines:

Not specified

GLP:

No

The study is acceptable

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl was tested in the Unscheduled DNA Synthesis (UDS) assay using primary cultures of rat hepatocytes. The concentrations tested were 0.001, 0.01, 0.033, 0.10, 0.33, 1.0, 2.0, 3.0, and 3.5 mM. The highest dose level was set based on solubility. Evaluations for UDS were conducted at all doses. For both the initial and independent repeat assays, the test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). 9,10-Dimethyl-1,2-benzanthracene and DMSO served as positive and vehicle controls, respectively. Benzo(a)pyrene was included in each trial to investigate its suitability as positive control. Hepatocyte cultures were exposed to their respective concentrations of the test substance (or positive or vehicle control), along with 5 µCi ³H-thymidine/mL, for approximately 18 hours. Mean net nuclear grain counts were obtained for each group from autoradiography slides. Cytotoxicity was assessed based on lactate dehydrogenase (LDH) concentration in the

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

Toxicology and metabolism

culture medium. A test substance was considered positive if the increase in average cytoplasmic grains was statistically significant when compared to the negative control response and if there was a statistically positive correlation between increasing concentrations of test substance and the average UDS response in the absence of a negative correlation between test concentration and average cytoplasmic grains. Statistical significance was judged at the 5% level.

Findings:

None of the concentrations of the test substance caused a significant increase in the mean net nuclear grain counts when compared to negative controls (). INF-5384-52 at 1.0 mM in trial 1, and at ≥ 2.0 mM in trial 1 and 2, was toxic as measured by release of lactate dehydrogenase into the treatment medium. Precipitation at the start of treatment was observed at ≥ 2.0 mM. The positive control 9,10-Dimethyl-1,2-benzanthracene, but not benzo(a)pyrene, induced an increase in UDS. Benzo(a)pyrene was added to study for investigative reasons and the failure to induce a positive response had no impact on the outcome of the study.

Table B.6.29: Summary of UDS assay

Treatment	Concentration (mM)	Average net nuclear photometer reading ^a	
		Initial assay	Repeat assay
DMSO	0	-1.7 (1.8) ^b	-5.0 (2.9) ^b
		-2.1 (1.9)	-2.8 (4.1)
Bensulfuron methyl	0.001	-4.8 (2.7)	-2.5 (3.1)
		-1.5 (3.4)	-6.7 (3.5)
	0.010	-3.9 (2.2)	-2.2 (2.6)
		-0.6 (3.4)	-4.9 (3.1)
	0.033	-1.3 (1.8)	-4.9 (4.0)
		-1.7 (3.1)	-4.4 (4.5)
	0.10	-5.5 (2.9)	-5.2 (3.0)
		-3.7 (2.2)	-8.7 (5.0)
	0.33	-3.7 (2.7)	-4.1 (3.4)
		-3.8 (2.4)	-2.0 (4.0)
	1.0	-1.0 (3.1)	-4.4 (2.5)
		-4.0 (3.2)	-3.0 (2.0)
	2.0	-3.6 (2.2)	-5.0 (2.8)
		-4.2 (1.7)	-3.5 (2.6)
	3.0	-2.4 (1.4)	-2.4 (2.2)
		-3.3 (3.3)	-3.6 (3.5)
	3.5	-4.9 (2.3)	-3.7 (2.4)
		-4.6 (4.3)	-3.7 (1.8)
DMBA	1.0	19.3 ^c (10.1)	27.2 ^c (15.3)
		19.8 ^c (13.1)	22.6 ^c (25.3)
B(a)P	0.05	-4.4 (2.1)	-1.9 (3.4)
		-3.1 (2.7)	-0.7 (2.8)

^aRepresents the counts on 2 slides per treatment; photometric reading were taken over 25 nuclei per slide were counted.

^bStandard deviation in parenthesis

^cStatistically significant

DMBA = 9,10-Dimethyl-1,2-benzanthracene; B(a)P = Benzo(a)pyrene; DMSO = dimethyl sulfoxide

Conclusion:

Bensulfuron methyl did not cause a significant increase in unscheduled DNA synthesis and was concluded to be negative in this assay.

Rapporteur Member State	Month and year	Active Substance (Name)
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Toxicology and metabolism

B.6.4.4 *In-vitro* mammalian cytogenicity test – sister chromatid exchange in Chinese hamster ovary (CHO) cells

22) *In vitro evaluation of INF-5384-52 for sister chromatid exchange in chinese hamster ovary (CHO) cells.*

Vlachos, D.A., 1986.

Cross reference IIA, 5.4.1./05

Guidelines:

USEPA 84-2

GLP:

Yes

The study is acceptable

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl was tested for induction of sister chromatid exchange (SCE) in the *in vitro* mammalian cytogenetic test using Chinese hamster ovary (CHO) cells both with and without an exogenous metabolic activation system (Aroclor[®] 1254-induced rat liver S9). Doses in the initial and independent repeat assays were 0.135, 0.270, 0.800, 1.350, and 2.700 mM. The highest dose level was set based on solubility of the test substance and cytotoxicity (cell cycle delay) test. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 272.2 mM. Mitomycin C and cyclophosphamide were used as the positive control for the non-activated and S9 activated studies, respectively. CHO cells were treated for 2 hours, and, after exposure to Colcemid[®], metaphase cells were harvested 26 – 28 hours following the initiation of treatment. Cells were evaluated for SCE. The test substance was considered to induce a positive response if the mean SCE frequency of one or more of the sample concentrations tested was significantly greater than that of the solvent control ($p \leq 0.05$) and the correlation between the mean SCE frequency and the concentration of the test sample is significantly greater than 0, where significance is judged at the 0.01 level.

Findings:

A statistically significant increase in SCE was observed in the nonactivated test system. This response was not seen in the S9 activated test system. There was no evidence of toxicity by the test compound.

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)
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Toxicology and metabolism		

Table B.6.30: Summary of sister chromatid exchange data

Treatment	Conc. mM	Trial 1		Trial 2	
		Mean # of SCE/metaphase (SEM)		Mean # of SCE/metaphase (SEM)	
		S9-	S9+	S9-	S9+
		24 hrs	24 hrs	24 hrs	24 hrs
DMSO	0	5.5 (0.1)	9.2 (0.1)	8.0 (0.2)	10.2 (0.0)
Bensulfuron methyl	0.135	5.6 (0.4)	10.1 (0.2)	8.5 (0.1)	9.7 (0.1)
	0.270	7.0 (0.1)	9.3 (0.0)	8.1 (0.0)	10.4 (0.0)
	0.800	5.5 (0.1)	8.8 (0.1)	8.9 (0.1)	10.5 (0.0)
	1.350	6.8 (0.2) ^b	10.0 (0.0)	11.5 (0.3) ^b	11.0 (0.1)
	2.700	7.9 (0.3) ^b	a	11.3 (0.0) ^b	10.9 (0.3)
MMC	2.5×10^{-5}	12.1 (0.2) ^b	ne	16.1 (0.2) ^b	ne
CP	0.009	ne	11.9 (0.0)	ne	17.1 (0.4)

MMC = mitomycin C; CP = cyclophosphamide; DMSO = dimethyl sulfoxide

^a cells could not be analysed due to poor staining^b statistically significant ($p \leq 0.01$; Student's t-test); statistical analyses based on combining results from trial 1 and 2

ne = Not evaluated

Conclusion:

Bensulfuron methyl was positive for sister chromatid exchange in the non-activated test system, and negative in the S9 activated test system in the in vitro mammalian cytogenetics test using Chinese hamster ovary cells.

B.6.4.5 In vivo studies in somatic cells**B.6.4.5.1 In-vivo bone marrow cytogenicity test – clastogenicity in rats**

23) In vivo bone marrow chromosome study in rats. Farrow, M.G., 1984.

Cross reference IIA, 5.4.2./01

24) In vivo bone marrow chromosome study in rats treated with INF-5384-52 (H# 14,793-02). Vlachos, D.A., 1987.

Cross reference IIA, 5.4.2./02

Guidelines:

US EPA Subdivision F, 84-2

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN F5384 technical

Batch: F5384-52

Purity: 95.9%

Bensulfuron methyl was evaluated for its ability to induce numerical and structural chromosome aberrations in the bone marrow of male and female Charles River Sprague-Dawley, CD[®] rats (15 rats/sex/group at each of 3 time

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points). Animals received 500, 1,500 or 5,000 mg/kg of body weight by single-dose oral gavage. The highest dose level was set based on the limit dose for this test system. The test substance was suspended in corn oil. Cyclophosphamide (CP, 40 mg/kg) was used as the positive control. The dosing factor was 15 mL/kg for all dose groups. In-life observations included clinical signs and body weight determinations. Two hours before sacrifice all animals received a single intraperitoneal injection of colchicine (2.0 mg/kg) to inhibit mitosis and arrest cells in metaphase. The animals were sacrificed and bone marrow metaphase slides were prepared approximately 6, 24, and 48 hours after dosing for the negative control and test substance treated dose groups. The positive control was included only at the 24-hour sacrifice point. When possible, 50 cells in metaphase per animal were examined for numerical and structural chromosome aberrations. The mitotic indices per 500 cells undergoing mitosis were also determined to assess toxic effects on the bone marrow.

Findings:

There were no statistically significant increases in the frequency of chromosome aberrations compared to vehicle (negative) control values (). In addition, there were no statistically significant differences in the mean mitotic indices in male or female mice administered bensulfuron methyl compared to the vehicle control. There were no test substance related biologically significant clinical signs or mortality in any dose group. No significant variance in mean body weight changes was observed for any of the dose groups at any time point.

Table B.6.31: Summary of chromosome aberration data

DPX-F5384-52 dose (mg/kg)	Sample time (hours)	Sex	Number of animals	Average number of mitoses per 500 cells ^a (S.D)		Average number of aberrations per cell ^a	Percent of cells with structural aberrations
Corn Oil	6	M + F	10	1.56	1.352	0.004	0.40
500	6	M + F	10	2.64	2.033	0.002	0.20
1500	6	M + F	10	1.56	1.339	0.002	0.22
5000	6	M + F	10	3.64	1.945	0.002	0.20
Corn Oil	24	M + F	10	1.78	1.160	0.002	0.2
500	24	M + F	10	2.48	2.365	0.007	0.67
1500	24	M + F	10	2.30	1.227	0.006	0.60
5000	24	M + F	10	1.52	1.251	0.002	0.20
Corn Oil	48	M + F	10	1.84	1.21	0.002	0.20
500	48	M + F	10	1.94	1.088	0.000	0.00
1500	48	M + F	10	1.94	1.523	0.004	0.40
5000	48	M + F	10	2.22	2.014	0.000	0.00
CP (20 mg/kg)	24	M + F	10	0.30	0.492	1.665 ^b	30.94 ^b

^a 205 metaphase cells analysed per sex/dose group

^b Statistically significant from control, $p < 0.05$, using Kruskal-Wallis nonparametric analysis of variance and non-parametric pair wise comparisons

S.D. = Standard deviation

CP = Cyclophosphamide

Conclusion:

Bensulfuron methyl did not induce an increase in numerical or structural chromosome aberrations in bone marrow cells of mice and is considered negative in this *in vivo* assay.

B.6.4.6 *In vivo* studies in germ cells

No *in vivo* studies in germ cells were conducted with bensulfuron methyl.

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Toxicology and metabolism

B.6.4.7 Summary of genotoxicity testing

A summary of the results of genotoxicity testing is given in .

Table B.6.32: Summary of genotoxicity studies

Type of study	Test system	Concentration range tested	Result	Reference
<i>In vitro</i> bacterial mutagenicity (Ames)	<i>Salmonella typhimurium</i>	0-25 µg/plate (with and without S-9)	negative	Arce, 1985; Rev. 1 1987
<i>In vitro</i> chromosome aberration (clastogenicity)	human lymphocytes	0.1 – 1.1 mg/mL (with and without S-9)	negative	Covell, 1988
<i>In vitro</i> mammalian cell mutagenicity (CHO/HGPRT)	CHO cells	0.5 – 4.0 mM (with and without S-9)	negative	Chromey, 1984
<i>In vitro</i> unscheduled DNA synthesis	rat primary hepatocytes	0.001 – 3.5 mM	negative	Chromey, 1984
<i>In vitro</i> sister chromatid exchange (cytogenicity)	CHO cells	0.135 – 2.700 mM (with and without S-9)	positive	Vlachos, 1986
<i>In vivo</i> chromosome aberrations	rat bone marrow	Male and female: 500, 1,500 or 5,000 mg/kg bw	negative	Farrow, 1984 Vlachos, 1987

Although a positive response occurred in one *in vitro* study in the absence of S9, the weight of evidence from other studies suggests that bensulfuron methyl does not cause genetic damage.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

The chronic toxicity and/or carcinogenicity of bensulfuron methyl were evaluated in rats, mice, and dogs. The studies in rats and mice are summarised below. The 1-year study in dogs is summarised under Point 5.3.2 of this document.

B.6.5.1 Oral 24-Month Study in Rats

25) *Long-term feeding and two-generation, four-litter reproduction study in rats with INF-5384 (Four Volumes). Stadler, J.G., 1986.*

Cross reference IIA, 5.5./04

Guidelines:

US EPA 83-5

Deviations: None

GLP:

Yes

The study is *acceptable*

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-38 and F5384-52

Purity: 95% and 95.9%, respectively

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- 26) *Combined chronic toxicity/oncogenicity study with IN F5384 long-term feeding and two-generation, four-litter reproduction study in rats supplemental appendix (Two Volumes). Slone, T.W., 1988a.*

Cross reference: IIA, 5.5./02

Guidelines:

USEPA 83-5

Deviations: Not applicable

GLP:

Yes

The study is *acceptable*

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-38 and F5384-52

Purity: 95% and 95.9%, respectively

- 27) *Combined chronic toxicity/oncogenicity study with IN-F5384 two-year feeding study in rats supplemental report (Supplement No. 2). Stula, E.F., 1988.*

Cross reference IIA, 5.5./05

Guidelines:

USEPA 83-5

Deviations: Not applicable

GLP:

Yes

The study is *acceptable*

Material and methods

Test substance: DPX-F5384 technical

Batch: F5384-38 and F5384-52

Purity: 95% and 95.9%, respectively

- 28) *Combined chronic toxicity/oncogenicity study with IN F5384 long-term feeding and two-generation, four-litter reproduction study in rats supplemental tables and appendix. Slone, T.W., 1988b.*

Cross reference IIA, 5.5/03

Guidelines:

USEPA 83-4

Deviations: Not applicable

GLP:

Yes

The study is *acceptable*

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

Toxicology and metabolism

Materials and methods:

Test substance: DPX-F5384 technical

Batch: F5384-38 and F5384-52

Purity: 95% and 95.9%, respectively

In a 2-year chronic toxicity, carcinogenicity feeding and 2-generation, 4-litter reproduction study, bensulfuron methyl was administered to male and female CrI:CD[®](SD)BR rats (approximately 80 rats/sex/concentration). Concentrations were 0, 50, 750, and 7500 ppm for male and female rats. The mean daily intakes for male rats were 0, 2.0, 30, and 309 mg/kg bw/day. The mean daily intakes for female rats were 0, 2.7, 40, and 405 mg/kg bw/day. Ten rats per group were sacrificed after approximately 1 year on study and all surviving rats were sacrificed after approximately 2 years on study. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, organ weights, and gross and microscopic pathology. A 2-generation, 4-litter reproduction sub-study was initiated approximately 3 months after the start of the feeding study with 20 rats per sex per group. During this substudy, rats were continued on their group's respective diets. Following the birth and weaning of the first generation (F₁ litters), these parental rats were returned to the combined chronic toxicity and oncogenicity study.

Findings:

General observations: No effects from the dietary intake of the test substance were seen in clinical observations neither in this study nor in the incidence of unscheduled mortalities. Mean body weights of 7500 ppm female rats were significantly lower than the mean body weights of the female control group from Days 438-602. In general, a number of groups had mean weight losses during the last several months of the study. These weight losses were considered to be age-related and not a result of intake of the test substance. However, the consistently lower weights for 7500 ppm females were considered to be a test substance-related effect. Food consumption was also lower for 7500 ppm females when compared to controls. Food efficiency values were similar for all groups within a sex.

Haematology, clinical chemistry, and urinalysis: Several clinical parameters for test substance-treated groups were significantly different from controls; however, only decreased haemoglobin in 7500 ppm males at the 24-month evaluation was considered to be test substance-related (Table B.6.40). No other test substance-related effects were observed in clinical chemical, haematological, or urinalysis parameters.

Table B.6.40: Two-year feeding study in rats: Haematological evaluation at 24 months

	0 ppm	50 ppm	750 ppm	7500 ppm
Males:				
Hb (g/dL)	15.2	15.0	15.2	13.2 ^a
Females:				
Hb (g/dL)	15.2	15.5	14.9	15.0

^aSignificantly different from control by the Dunnett's criteria, p < 0.05.

Hb Haemoglobin

Gross pathology, organ weights, histopathology: No remarkable gross pathology changes were observed in test substance-treated rats. Several statistical differences in mean organ weights were observed at the 12-month and 24-month sacrifices; however, these differences were considered to be secondary to reduced body weight compared to the controls. Histological changes were observed in the livers of 7500 ppm males and females (Table B.6.41). These microscopic changes consisted of centrilobular hepatocellular hypertrophy, with margination of the basophilic

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cytoplasm and infrequent cytoplasmic fatty change. A higher incidence of liver effects was evident at the one-year sacrifice than at the end of the study, which indicates that these changes were not progressive. There were no test substance-related neoplastic lesions.

Table B.6.41: Two-year feeding study in rats: Incidences of non-neoplastic microscopic effects at the 24-month sacrifice

Bensulfuron methyl (ppm):	0	50	750	7500
Number of rats/group (m/f):	25/33	34/29	31/36	23/35
Males:				
Liver				
Angiectasis, sinusoidal, focal	3 ^a	4	1	2
Arteritis	1	0	0	0
Degeneration, cystic, hepatocellular, focal	9	11	13	8
Fatty change, diffuse	0	0	2	1
Fatty change, focal	3	1	2	6
Fatty change, periportal	4	6	2	2
Hematopoiesis, focal	0	1	0	0
Hemosiderosis	0	2	0	0
Hepatocellular alteration, focal	3	11	9	1
Hepatocellular nodule (hyperplasia)	2	1	0	0
Histiocytosis, focal	0	1	2	0
Hyperplasia/inflammation/fibrosis, bile duct	14	25	20	16
Hypertrophy, hepatocellular, centrilobular	0	0	0	11 ^b
Margination, cytoplasmic, basophilia, hepatocyte, centrilobular	0	0	0	13 ^b
Necrosis, focal	0	3	0	0
Females:				
Liver				
Angiectasis, sinusoidal, focal	0	3	4	2
Atrophy, hepatocellular, diffuse	0	0	0	0
Cyst (s), bile duct	2	0	0	0
Degeneration, cystic, hepatocellular, focal	2	1	1	0
Fatty change, centrilobular	1	0	0	1
Fatty change, diffuse	1	3	0	3
Fatty change, focal	2	0	3	1
Fatty change, periportal	12	13	10	13
Fibrosis, centrilobular	0	0	0	1
Hematopoiesis	3	3	3	5
Hemosiderosis	1	0	1	2
Hepatocellular alteration, focal	6	2	8	1
Hepatocellular nodule (hyperplasia)	0	0	0	0
Hyperplasia/inflammation/fibrosis, bile duct	17	17	11	17
Hypertrophy, hepatocellular, centrilobular	0	0	0	6 ^b
Margination, cytoplasmic, basophilia, hepatocyte, centrilobular	0	0	0	7 ^b
Necrosis, focal	0	0	0	1

^aNumber of organs with microscopic change.

^bStatistically significant by the Fisher's Exact Test criteria, $p < 0.05$.

Reproduction Substudy

No test substance-related effects on body weight, weight gain, food consumption, food efficiency, clinical observations, or mortalities were seen in the F₀ or F₁ parental rats fed bensulfuron methyl in the diet for 90 days.

No effects were seen in reproduction parameters that could be attributed to dietary intake of bensulfuron methyl.

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Toxicology and metabolism

There were no test substance-related clinical signs noted in either F₁ pups or F₂ pups. There were no test substance-related effects on mean weights for F_{1a} pups produced during the first mating of the F₀ generation or for the F_{1b} pups.

There were no test substance-related effects on litter weights in the F_{2a} or F_{2b} generation.

In the F_{1a} generation, although no statistically significant differences were evident, the number of pups born alive, the number of pups alive at 24 hours, and the gestation index were slightly lower for 7500 ppm litters than for control. A statistically significant difference in the 0-4 day viability index occurred in litters from the 50 ppm group. At least 1 pup died during the first 24 hours in five of the sixteen 750 ppm litters. No significant differences were evident for any of the other pup counts or reproduction or lactation indices. In the F_{1b} generation, no significant differences in pup mortality were apparent when test substance-treated groups were compared to control. No statistically significant differences were apparent in any of the reproduction or lactation indices for the F_{2a} generation. In the F_{2b} generation, litter weights for the F_{2b} pups from the 50 ppm group were lower than controls. None of the effects seen in the F_{1a} or F_{1b} or in the F_{2a} or F_{2b} generations was attributed to intake of bensulfuron methyl.

In the F_{2b} weanlings sacrificed for pathological evaluation, there were no gross or histopathological changes attributed to dietary intake of bensulfuron methyl and no statistically significant differences in organ weights.

Conclusion: The NOAEL in the 2-year feeding study in rats was 750 ppm for males and females (30 and 40 mg/kg/day, respectively). This NOAEL was based on lower mean body weights in 7500 ppm females, anaemia at 24 months in 7500 ppm males and histopathological changes in the liver of 7500 ppm males and females. Under the conditions of this study, bensulfuron methyl was not a carcinogen. The results of the reproduction substudy were not used to establish the NOAEL since they were superseded by a multigeneration reproduction study reported in Point 5.6.1 of this document.

B.6.5.2 Oral 24-month study – mice

29) *DPX-F5384: 24-month oral chronic toxicity and oncogenicity study in mice (10 Volumes). Maita, K., 1985. Cross reference IIA, 5.5/01*

Guidelines:

USEPA Subdivision F 83-2

Deviations: None

GLP:

Yes

The study is *acceptable*

Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5348-52

Purity: 95.9%

In a 24-month combined chronic toxicity/carcinogenicity feeding study, bensulfuron methyl was administered to male and female SPF ICR (Crj:CD-1) mice (approximately 92 mice/sex/concentration) at concentrations of 0, 10, 150, 2500, and 5000 ppm. The mean daily intakes in male mice were 0, 0.87, 13.35, 226, and 455 mg/kg bw/day. The mean daily intakes in female mice were 0, 0.93, 13.63, 227, and 460 mg/kg bw/day. After 52, 78, and 104 weeks

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Toxicology and metabolism

of treatment, 10 mice/sex/dose were subjected to urinalysis, haematology and blood biochemistry analyses. Ten mice/sex/dose were sacrificed at approximately 52 and 78 weeks on test and all surviving mice were sacrificed after 104 weeks on test and subjected to gross and histopathology. Parameters evaluated included body weight, body weight gain, food and water consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology.

Findings:

General observations: There were no test substance-related clinical signs noted, and no test substance-related effects on mortality. There were no test substance-related effects on body weights, body weight gains, food consumption or food efficiency. Water consumption was slightly lower among males and females at 5000 ppm (Table B.6.42). For 5000 ppm males, average water consumption was 89% of control value (Table B.6.42). Average mean water consumption for 5000 ppm females was 85% of control and sporadically reached statistical significance from approximately weeks 4 to 68. There were no effects on water consumption in 2500 ppm males and females and below.

Table B.6.42: 24-month feeding study in mice: Average water consumption over 104 weeks

	0 ppm	10 ppm	150 ppm	2500 ppm	5000 ppm
Males:					
Water consumption (mL/day)	7.5	7.3	7.3	6.9	6.7
Females:					
Water consumption (mL/day)	6.5	6.5	6.5	6.2	5.5

Haematology and clinical chemistry: Although there were significant changes in some parameters in the treated groups of both sexes after 52 weeks of treatment, no dose-related effect relationships were observed. At other evaluation time points, no significant fluctuations of the values were recorded in the treated groups of both sexes. A statistically significant decrease in glutamic oxaloacetic transaminase was noted in 2500 and 5000 ppm males at the 52 week evaluation. This decrease was not observed at subsequent evaluations. There were no significant deviations from control noted in males at 150 ppm and below. There were no clinical chemistry findings in any female group.

Urinalysis: Specific gravity of urine was significantly increased in 5000 ppm males at the 52 week interim evaluation, but no changes were noted in this parameter at other evaluation time points. Significant fluctuations were also recorded in some parameters in the treated groups of both sexes at each evaluation time point, however, these fluctuations showed no relationship with the dose levels or duration of treatment.

Gross pathology, organ weights, and histopathology: Test substance-related gross findings in 5000 ppm males included an increased incidence of nodules and/or masses in the liver at 52 weeks. However, this finding was not noted in subsequent evaluations. In 5000 ppm females, there was a statistically significant increased incidence of thymic enlargement and intra-abdominal nodules or masses. There were no gross findings noted in animals of either sex at 2500 ppm and below. There were no statistically significant changes in organ weight noted in any treated group of male mice. Significant elevations of relative liver weights at the 52 week evaluation were noted in 5000 ppm female mice (Table B.6.43). Absolute and relative liver weights were statistically increased in 5000 ppm females at the 24-month evaluation. Absolute and relative liver weights of males were also increased. However, although the magnitude of this effect was slightly greater than that in females, it was not statistically significant.

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Toxicology and metabolism

There were no test substance-related neoplastic lesions noted in either sex at any dose level. Statistically significant fluctuations were noted in some non-neoplastic lesions in the treated groups when compared to control (Table B.6.44). Males in the 5000 ppm group had a significantly higher incidence of hepatocellular swelling at the 52 and 104 week sacrifices. Since this effect is an adaptive response to metabolism of a xenobiotic and was not associated with any other neoplastic or non-neoplastic changes in the liver, it was not considered to be adverse. The incidence of renal cortical cysts in 5000 ppm females was significantly increased at the 52 week sacrifice. However, the incidence was similar to control at the 78- and 104-week sacrifices, and no other test substance-related renal lesions were observed. Therefore, the increased incidence of renal cortical cysts in 5000 ppm females at the 52 week sacrifice was not considered to be adverse. Renal pelvic dilation in the kidney was increased in 10, 150, 2500, and 5000 ppm females at the 104 week sacrifice; however, a dose response-relationship was not evident, and the finding was not associated with any other change in renal morphology or clinical chemistry or urinalysis parameters. Therefore, the increased incidence was not considered to be adverse.

Table B.6.43: 24-month feeding study in mice: Organ weights at the 24-month sacrifice

	0 ppm	10 ppm	150 ppm	2500 ppm	5000 ppm
Males:					
Absolute liver weight (g)	3.08	3.36	3.07	3.75	4.54
Relative ^a liver weight	6.46	6.49	6.14	7.54	9.43
Females:					
Absolute liver weight (g)	2.04	1.95	2.29	2.42	2.42 ^b
Relative ^a liver weight	4.60	4.51	5.12	5.07	5.64 ^c

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by the Dunnett's criteria, $p < 0.05$.

^c Significantly different from control by the Dunnett's criteria, $p < 0.01$.

Conclusion:

Based on the absence of persistent, adverse effects at any dietary concentration in either males or females, the NOAEL was considered to be 5000 ppm (455 and 460 mg/kg/day in males and females, respectively). Under the conditions of this study, bensulfuron methyl was not a carcinogen.

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Toxicology and metabolism

Table B.6.44: 24-month feeding study in mice: Incidences of non-neoplastic microscopic effects at the 24-month sacrifice

Bensulfuron methyl (ppm):	0	10	150	2500	5000
Number of mice/group:	92	92	92	92	92
Males:					
Liver					
Centrilobular hepatocellular fatty change	7 ^a	8	11	6	8
Focal hepatocellular fatty change	1	0	0	1	3
Diffuse hepatocellular fatty change	0	1	0	1	0
Centrilobular hepatocellular necrosis	0	0	0	1	3
Focal hepatocellular necrosis	4	3	5	2	1
Massive hepatocellular necrosis	4	1	1	1	1
Hepatocellular swelling (centrilobular)	0	0	0	1	7 ^c
Hepatocellular swelling (diffuse)	2	0	0	1	1
Foci of cellular alterations (basophilic)	1	0	3	6	1
Foci of cellular alterations (acidophilic)	2	0	1	3	4
Foci of cellular alterations (clear cell)	0	0	0	1	0
Increased brown pigment deposition (stellate cells)	1	2	1	2	3
Increased extramedullary hematopoiesis	0	2	2	2	2
Kidneys					
Increased brown pigment – proximal tubular cells	0	0	0	1	0
Hyaline droplet degeneration – proximal tubular cells	0	0	0	0	1
Tubular cell swelling	0	0	0	2	0
Tubular atrophy	22	23	35 ^b	29	33
Cortical cyst	15	14	14	10	10
Increased extramedullary hematopoiesis	0	0	0	0	1
Mineralisation	0	0	1	0	2
Pelvic dilatation	24	29	25	16	15
Arteritis	8	8	5	2 ^b	1 ^b
Females:					
Liver					
Focal hepatocellular fatty change	1 ^a	0	1	0	0
Diffuse hepatocellular fatty change	6	5	2	3	6
Focal hepatocellular necrosis	5	2	8	5	12
Massive hepatocellular necrosis	0	0	1	0	0
Hepatocellular swelling (diffuse)	0	0	0	1	2
Foci of cellular alterations (acidophilic)	4	0	2	2	3
Incr. brown pigment deposition – stellate cells	1	1	1	1	5
Increased extramedullary hematopoiesis	11	6	6	2 ^c	13
Advanced Autolysis	0	0	1	1	0
Kidney					
Increased brown pigment – proximal tubular cells	1	0	1	0	4
Hyaline droplet degeneration – proximal tubular cells	2	4	1	1	2
Tubular cell vacuolation	0	0	3	0	0
Tubular dilatation	1	0	0	0	0
Tubular atrophy	10	10	13	15	17
Cortical cyst	7	13	8	7	13
Increased extramedullary hematopoiesis	1	0	1	0	1
Mineralisation	1	0	1	0	0
Pelvic dilatation	2	8 ^b	9 ^b	9 ^b	6
Arteritis	2	1	1	1	0

^aNumber of organs with microscopic change.

^bStatistically significant by the Fisher's Exact Test criteria, p <0.05

^cStatistically significant by the Fisher's Exact Test criteria, p <0.01

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Toxicology and metabolism

B.6.5.3 Oral 1-year study - dogs

The 1-year study in dogs is summarised under Point 5.3.2.4 of this document.

B.6.5.4 Summary of long-term toxicity and carcinogenicity

The no-observed-adverse-effect levels from the chronic toxicity studies with bensulfuron methyl are summarised in Table B.6.45.

Table B.6.45: Summary of chronic toxicity studies

Type of study	Species	Dose range tested	NOAEL		Reference
			ppm	mg/kg/d	
Oral (feeding), 2-year	rat	0, 50, 750, 7500 ppm	750	m: 30 f: 40	Stadler, 1986
Oral (feeding), 18-month	mouse	0, 10, 150, 2500, 5000 ppm	5000	m: 455 f: 460	Maita, 1985

Bensulfuron methyl was not carcinogenic in rats or mice.

The NOAEL levels for chronic toxicity in rats were based on body weight and nutritional decrements in 7500 ppm females, decreased haemoglobin in 7500 ppm males, and increased incidences of centrilobular hypertrophy with margination of the basophilic cytoplasm and cytoplasmic fatty change.

The NOAEL for chronic toxicity in mice was based on absence of persistent adverse effects at any dietary concentration.

Conclusion:

Based on the results of chronic feeding studies in rats and mice, bensulfuron methyl is not a carcinogen, and the lowest NOAEL of approximately 30 mg/kg/day is based on decreased body weight in 7500 ppm female rats, decreased haemoglobin in 7500 ppm male rats, and histopathological changes in the livers of male and female rats administered 7500 ppm.

B.6.6 Reproductive and developmental toxicity (IIA 5.6)

A two-generation reproduction study was conducted in rats with bensulfuron methyl. Developmental studies in the rat and rabbit were also conducted.

B.6.6.1 Multigeneration study - rats

30) *Reproductive and fertility effects with IN-F5384-122 multigeneration reproduction study in rats. Mullin, L.S., 1989.*

Cross reference IIA, 5.6.1./01

Guidelines:

US EPA 83-4

Deviations: The cervix was not collected. However, in the absence of effects on fertility in earlier studies, this is not expected to impact study quality.

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Toxicology and metabolism

GLP:

Yes

*The study is acceptable.*Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-122

Purity: 97.3%

In a two-generation reproduction study, bensulfuron methyl was administered in the diet to male and female CrI:CD[®]BR rats (30 rats/sex/concentration for both the F₀ and F₁ generations). Concentrations were 0, 250, 7500, or 20,000 ppm. After 70 days on test, the F₀ rats were bred within their treatment groups to produce F₁ litters. The F₁ rats were bred within their respective treatment groups to produce F₂ litters at least 105 days after weaning. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, ophthalmology, reproductive indices, litter and pup parameters, organ weights, and gross and microscopic pathology.

Findings:F₀ and F₁ Parental Rats

General observations: There were no significant test substance-related differences in body weight or weight gain between control test substance-treated F₀ males or F₀ or F₁ females at any time during the study. Body weights and weight gains of F₁ males in the 20,000 ppm group were approximately 7 to 8% lower than those of controls during the study (). Body weight was also significantly decreased in the 7500 ppm F₁ males during test days 13-77, and test day 91. Food consumption of F₀ males in the 20,000 ppm group was significantly greater than control male food consumption in the pre-mating period; however, this is not considered a test substance-related effect. Food consumption of F₁ males and F₀ and F₁ females was comparable to controls throughout the study. The mean daily intakes during premating phases for 250, 7500, and 20000 ppm F₀ and F₁ males were 14.5-19.5, 426.2-594.8, and 1201.0-1589.4 mg/kg bw/day, respectively. The corresponding premating mean daily intakes for F₀ and F₁ females were as follows: 17.1-22.3, 515.3-694.9, and 1404.5-1874.0 mg/kg bw/day, respectively. No clinical signs observed in any rats during the study were attributed to dietary administration of the test substance. No parental generation rats died during the study. No test substance-related eye lesions were found in F₀ generation rats. Eyes of the F₁ generation rats were not examined.

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Toxicology and metabolism

Table B.6.46: Two-generation reproduction study: Body weights, of F₁ male parental rats

Days on Test	0 ppm	250 ppm	7500 ppm	20,000 ppm
F ₁ Males:				
Day 0	57.9	55.8	54.3	53.7
Day 6	94.0	92.3	89.5	88.6
Day 13	152.4	148.3	142.1 ^a	143.2 ^a
Day 20	217.2	209.7	201.0 ^a	204.5 ^a
Day 27	278.7	271.3	260.4 ^a	263.2 ^a
Day 35	343.9	330.6	322.9 ^a	319.4 ^a
Day 42	392.0	383.6	370.6 ^a	364.8 ^a
Day 49	438.1	428.9	411.7 ^a	406.6 ^a
Day 56	473.3	464.9	443.8 ^a	435.4 ^a
Day 63	498.4	490.3	469.1 ^a	461.7 ^a
Day 70	526.3	516.5	494.2 ^a	486.3 ^a
Day 77	551.4	541.5	517.7 ^a	502.0 ^a
Day 84	568.4	560.8	540.2 ^a	523.8 ^a
Day 91	586.4	578.4	553.9 ^a	538.8 ^a
Day 98	598.2	592.0	567.8	550.9 ^a
Day 105	613.0	604.0	582.9	564.9 ^a
Day 112	624.5	620.9	598.3	572.0 ^a
Day 119	628.7	618.9	598.9	577.2 ^a
Day 126	636.5	634.0	611.5	591.3 ^a
Day 133	650.3	647.2	620.6	601.4 ^a
Day 140	663.0	665.5	634.6	610.8 ^a

^a Significantly different from control by the one way ANOVA and Dunnett's criteria, p < 0.05.

Reproductive parameters: There were no differences attributed to test substance administration in mating or fertility indices or gestation length in either generation. There were no test substance-related effects on litter size or survival in either generation.

Gross pathology, organ weights, and histopathology of the parental animals: There were no significant differences between control and test substance-treated groups in either relative or absolute testes weights of F₀ or F₁ males. Final body weights of F₁ adult males in the 20,000 ppm group were significantly lower than controls. There were no increases in any incidences of gross alterations in F₀ or F₁ adults. No test substance-related microscopic changes were found in the reproductive systems or pituitary glands of F₀ or F₁ adults.

F₁ and F₂ litter and pup data

General observations: There were no increases in any incidences of gross alterations in F₁ or F₂ found dead, culled, or weaned pups.

Litter weight, size, and pup survival: From approximately post partum day 4 through 21, the mean pup weights of the 7500 and 20,000 ppm F₁ litters ranged from 4 to 7% lower than mean F₁ control pup weights. However, this effect was not observed in the second generation litters and as well as it was not observed in an earlier rat reproduction study summarised under Point 5.5 of this document.

Litter size and pup survival of the F₁ offspring were similar to controls at birth and throughout lactation. In the F₂ pups, the sex ratio of the 7500 ppm rats was lower than controls at birth and consequently there were significantly fewer male pups and significantly more F₂ female pups throughout lactation at that concentration. This shift in sex ratio is not considered a test substance-related effect as it was not observed within the 20,000 ppm group. Rather it is considered to be due to the high sex ratio (greater number of males) born in the control litters of this generation. The 0-4 day viability of the 20,000 ppm F₂ litters was statistically lower than that of controls (98.0 vs. 99.7% for controls)

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98% viability is considered a parameter only marginally deviated by the control and so it is considered neither test substance-related or biologically significant. Presumably the 0-4 day viability of the 20,000 ppm group resulted significant at the statistical analysis because of the high survival rate of the controls.

Table B.6. 47: Two-generation reproduction study: Combined male and female pup weights for F₁ and F₂ generations

Days on Test	0 ppm	250 ppm	7500 ppm	20,000 ppm
F ₁ Post partum Day				
Day 0	6.6	6.5	6.3 ^a	6.4
Day 4 preculling	10.6	10.6	9.9	10.3
Day 4 postculling	10.6	10.6	10.0	10.3
Day 7	17.5	17.2	16.5 ^a	16.8
Day 14	35.3	34.8	33.6 ^a	33.5 ^a
Day 21	56.3	54.6	52.9	52.4
F ₂ Postpartum Day				
Day 0	6.5	6.1	6.5	6.5
Day 4 preculling	10.2	9.6	10.3	10.2
Day 4 postculling	10.2	9.6	10.3	10.3
Day 7	16.6	14.9 ^a	16.3	16.2
Day 14	33.3	30.6 ^a	32.1	31.7
Day 21	52.1	49.6	52.4	51.8

^a Significantly different from control by the Mann-Whitney U test at $p < 0.05$.

Gross pathology, organ weights, histopathology of pups and weanlings: F₂ weanlings in the 7500 and 20,000 ppm groups had heavier relative liver weights compared to controls. No test substance-related microscopic changes were found in any of the livers or other organs examined for F₂ weanlings. Therefore, the increased liver weight in 7500 and 20,000 ppm F₂ weanlings was not considered to be adverse or biologically significant.

Conclusion:

Reproduction NOAELs

Parental toxicity:	250 ppm (19.5 mg/kg/day for F ₁ males) based on slightly lower body weights in F ₁ adult males at 7500 and 20,000 ppm
Reproduction and fertility:	≥20,000 ppm [1404.5 – 1874.0 mg/kg bw/day for F ₀ and F ₁ dams, respectively] based on the absence of effects in the F ₀ and F ₁ generation at 20,000 ppm
Pup growth and development:	250 ppm (22.3 mg/kg bw/day for F ₁ dams) based on lower body weights in F ₁ litters at 7500 and 20,000 ppm

B.6.6.2 Developmental toxicity studies

Results of developmental toxicity studies with bensulfuron methyl by gavage (in rats and rabbits are summarised below.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

B.6.6.2.1 Developmental toxicity study - rats

31) *Developmental toxicity study of INF-5384-38 by gavage in the rat. Lamontia, C.L., 1999.*

Cross reference IIA, 5.6.2./01

Guidelines:

Not specified in the report

Deviations: Not applicable

GLP:

Yes

The study is acceptable.

Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-38

Purity: 95%

In a developmental toxicity study, bensulfuron methyl was administered by oral gavage to mated CrI:CD[®](SD)BR female rats (25/dose group) on gestation days 7-16. Gavage doses in stripped corn oil vehicle were 0, 50, 500, and 2000 mg/kg/day. The dose volume was 5 mL/kg. Parameters evaluated in dams were body weight, body weight gain (absolute and adjusted for the products of conception), food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, and skeletal malformations and variations.

Findings:

Maternal effects

General Observations: There were no test substance-related clinical signs observed at any dose level during the course of the study. Test substance-related mortality did not occur during the course of this study. There were no test substance-related effects on body weights, body weight gains, or food consumption.

Reproductive outcomes: There were no adverse test substance-related effects on reproductive parameters during the course of this study.

Gross pathology: No test substance-related gross lesions were observed at necropsy.

Foetal effects

The mean body weights of foetuses in the 50 and 2000 mg/kg groups were significantly lower than the controls, but a dose-response was not demonstrated, and the differences between the groups were minimal (). Therefore, the decreased body weights were not considered to be test substance related. No test substance-related increases occurred in the incidence of total malformations or in any specific type of external, visceral, or skeletal malformation. The incidence of total variations was significantly increased among 500 and 2000 mg/kg foetuses. The incidence of each type of variation (developmental and retarded development) was not test substance related, although the incidence of developmental variations was significantly increased among 2000 mg/kg foetuses. The incidence of lumbar ribs was increased among foetuses from the highest dose group and appeared dose-related (1.82%, 3.19%, 4.8% respectively in low, intermediate, high dose groups vs 1.97% in controls). The incidence of incompletely ossified hyoid bones, a variation due to retarded development, was higher than control values in foetuses at

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500 mg/kg and above. However, the incidence in the control group (9.3%) was lower than the historical control range (13.8-20.7%), and a dose-response relationship was not evident. The incidence of incompletely ossified sternums was increased among foetuses from the 2000 mg/kg group but there was no dose-response for percent affected. These changes were deemed singularly not toxicologically relevant, test substance-related and/or adverse but, as they occurred significantly at the highest dose level, they could be considered, together to the minimal reduction of foetal body weight, as a sign of embryo/foetal toxicity.

Table B.6.48: Developmental toxicity study in rats: Foetal parameters

	0 mg/kg	50 mg/kg	500 mg/kg	2000 mg/kg
Foetal Weight	3.6	3.4 ^a	3.5	3.5 ^a
Incidence of extra rib ossification centre	6/2 (2%)	5/3 (2%)	10/7 (3%)	16/8 ^a (5%)
Incidence of partially or unossified sternebrae	88/20 (29%)	121/19 (44%)	145/20 (46%)	156/23 ^a (47%)
Incidence of partially or unossified hyoid	14/9 (9.3%)	23/12 (17.5%)	35/15 ^a (23%)	31/17 ^a (20%)

^a Significantly different from control by the Dunnett's criteria, $p < 0.05$.

The first number is number of pups affected, the second number is number of litters affected, and the third number is percent of pups affected.

Conclusion:

NOAEL: ≥ 2000 mg/kg for both maternal effects and teratogenicity, the highest dose tested; 500 mg/kg for embryo/foetal toxicity.

B.6.6.2.2 Developmental toxicity study - rabbits

32) INF-5384. Developmental toxicity study in rabbits dosed by gavage on days 7-19 of gestation. Zellers, J.E., 1985.

Cross reference IIA, 5.6.2./02

Guidelines:

USEPA 83-3

Deviations: None

GLP:

Yes

The study is acceptable.

Material and methods

Test Substance: DPX-F5384 technical

Batch: F5384-80

Purity: 99%

33) Teratogenicity study of INF-5384 in rabbits dosed by gavage on days 7-19 of gestation. Zellers, J.E., 1987.

Cross reference IIA, 5.6.2./03

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

Guidelines:

EPA Subdivision 83-3

Deviations: None

GLP:

Yes

The study is acceptable.Materials and Methods:

Test substance: DPX-F5384 technical

Batch: F5384-80

Purity: 99%

In a developmental toxicity study, bensulfuron methyl was administered by oral gavage to time-mated Hra:(NZW)SPF female rabbits (20/dose group except control which was 22/dose group) on gestation days 7-19. Gavage doses in 0.5% methyl cellulose vehicle were 0, 30, 300, or 1500 mg/kg. The dose volume was 5 mL/kg. Parameters evaluated in dams were body weight, body weight gain (absolute and adjusted for the products of conception), food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, and skeletal malformations and variations.

Findings:Maternal effects

General Observations: In the 1500 mg/kg group, the incidences of red discharge on the cage board and stained tail were increased. These increases were associated with two females that had total resorptions, 1 female that aborted, and two pregnant animals found dead in the 1500 mg/kg group. Except for these and other associated findings, no test substance-related clinical signs were observed. No other test substance-related mortality during the course of this study. For the treatment period (Days 7-20G), only the 1500 mg/kg group experienced body weight changes significantly different from control (). This group had a net body weight loss (due to reduced gains for Days 7-16G and a significant loss for Days 16-20G) in comparison to equivalent net gains in all other groups. During the immediate post-treatment period (Days 20-24G), no significant difference in body weight gains were observed, but during the later stages of gestation (Days 24-29G), significantly reduced gains were observed at the 30 and 1500 mg/kg levels compared to controls. No dose response relationship was evident for the later interval. Rabbits administered 1500 mg/kg had significantly decreased food consumption compared to controls over Days 7 to 20G and for the immediate post-treatment period (Days 20-24G) compared to controls.

Table B.6.49: Developmental toxicity study in rabbits: Maternal body weight changes

	0 mg/kg	30 mg/kg	300 mg/kg	1500 mg/kg
Days 7-20	140.0	132.7	137.1	-89.8 ^a
Days 20-24	61.6	41.0	47.6	58.6
Days 24-29	108.8	14.4 ^a	78.0 ^a	28.5 ^a

^a Significantly different from control by the Dunnett's criteria, $p < 0.05$.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

Table B.6.50: Developmental toxicity study in rabbits: Food consumption

	0 mg/kg	30 mg/kg	300 mg/kg	1500 mg/kg
Days 7-20	139.1	141.0	138.6	110.5 ^a
Days 20-24	146.8	130.6	143.8	104.0 ^a
Days 24-29	135.8	110.5	130.2	106.9

^a Significantly different from control by the Dunnett's criteria, $p < 0.05$.

Reproductive outcomes: Except for a decrease in the mean number of corpora lutea in the 300 mg/kg dose group, no significant differences in reproductive parameters were observed among the control and test substance-treated group. In the 1500 mg/kg group, two females had total resorptions and one female aborted. No other females had total resorptions or abortions. For the females with live foetuses at scheduled sacrifice, no significant differences in the mean number of implantations, live foetuses, or resorptions were observed.

Table B.6.51 Developmental toxicity study in rabbits: Maternal reproductive effects

Parameters Observed	0 mg/kg	30 mg/kg	300 mg/kg	1500 mg/kg
Mean corpora lutea	11.1	10.4	9.3 ^a	11.0

^a Significantly different from control by the Mann-Whitney U criteria, $p < 0.05$.

Gross pathology: No test substance-related gross lesions were observed at necropsy.

Foetal effects

The mean female foetal weight for the high dose group was significantly lower than controls (). Mean female foetal weights for the 30 and 300 mg/kg groups were not affected. No significant differences in mean female foetal weight or combined foetal weight were observed between the control and test substance-treated groups. No significant difference in the rates of malformations or variations, variations due to retarded development, or both combined were observed between the control and test substance-treated groups.

Table B.6.52: Developmental toxicity study in rabbits: Mean foetal weight

Parameters Observed	0 mg/kg	30 mg/kg	300 mg/kg	1500 mg/kg
Total Foetal Weight (g)	50.79	50.34	50.26	43.17
Male Foetal Weight (g)	50.59	49.49	49.23	43.36
Female Foetal Weight (g)	50.26	49.00	48.88	38.66 ^a

^a Significantly different from control by the Mann-Whitney U criteria, $p < 0.05$.

Conclusion:

NOAEL: 300 mg/kg for both maternal and foetal effects. The maternal NOAEL was based on decreased body weights and food consumption, and on the incidence of spontaneous deaths at 1500 mg/kg. The foetal NOAEL was based on increased total resorptions and reduced foetal weights at 1500 mg/kg.

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

Toxicology and metabolism

B.6.6.3 Summary of reproduction and developmental studies

Table B.6. 53 Summary of reproduction and developmental studies

Type of study	Species	Doses/concentrations tested	NOAEL	Reference
Multigeneration reproduction	Rat	0, 250, 7500, 20,000 ppm in the diet	Parental: 250 ppm [M - 19.5 mg/kg bw/day] Repro/Fertility: $\geq 20,000$ ppm [1404.5 – 1874.0 mg/kg bw/day for F ₀ and F ₁ dams] Pup: 250 ppm [22.3 mg/kg bw/day for F ₁ dams]	Mullin, 1989
Developmental	Rat	0, 50, 500, 2000 mg/kg/day in corn oil by gavage	Maternal: ≥ 2000 mg/kg Foetal: 500 mg/kg	Lamontia, 1984
Developmental	Rabbit	0, 30, 300, 1500 mg/kg/day in methyl cellulose by gavage	Maternal: 300 mg/kg Foetal: 300 mg/kg	Zellers, 1987

In the two-generation reproduction toxicity study with bensulfuron methyl the NOAEL for parental toxicity was 250 ppm (19.5–22.3 mg/kg bw/day) based on slight reductions in body weight in F₁ adult males at 7500 ppm and above. The NOAEL for reproductive and fertility effects was 20,000 ppm (1404.5–1874.0 mg/kg bw/day), based on the absence of any effects at any dietary concentration. The NOAEL for effects on pup growth and development was 250 ppm (22.3 mg/kg bw/day for F₁ dams), based on a reduction in the body weight of F₁ pups at 7500 ppm and above. Developmental toxicity studies were conducted with bensulfuron methyl in rats and rabbits. Bensulfuron methyl was not teratogenic. In the developmental toxicity study in rats, the NOAEL for maternal effects was ≥ 2000 mg/kg/day based on the absence of maternal toxicity at 2000 mg/kg bw/day. The foetal NOAEL was 500 mg/kg/day, based on the adverse effects on pup weight, and the presence of variations in pups at 2000 mg/kg/day. In the developmental study conducted in rabbits, both the maternal and foetal NOAEL was 300 mg/kg/day based on death of females dosed with 1500 mg/kg/day and on the decreased maternal body weight and food consumption, and on total resorptions and decreased pup body weight at 1500 mg/kg bw/day.

B.6.7 Delayed neurotoxicity (IIA 5.7)

A delayed neurotoxicity study was not required for bensulfuron methyl. None of the acute, subchronic, chronic, or reproductive studies conducted with bensulfuron methyl in rats, mice, rabbits, or dogs demonstrated any effects suggestive of delayed neurotoxicity. No clinical signs of abnormal behaviour in any study have been noted, and no morphological changes in nervous system tissues in any study or species have been noted. Therefore, bensulfuron methyl does not appear to present a neurotoxic hazard.

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

Toxicology and metabolism

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity studies of metabolites

B.6.8.1.1 Acute oral toxicity of metabolites

34) *Approximate lethal dose (ALD) of INB-6895-2 in rats. Fisher, G.A., Sarver, J., 1985b.*

Cross reference IIA, 5.8.1/03

Guidelines:

Not specified

Deviations: Not applicable

GLP:

Not specified

*The study is **acceptable***

Materials and methods:

Test substance: IN-B6895-2 technical metabolite

Batch: B6895-2

Purity: 99.4%

IN-B6895-2, suspended in corn oil, was administered by single-dose oral gavage to one male rat (CrI:CD[®] (SD)BR) per dosage, at dosages of 3,400-11,000 mg/kg. Surviving animals were weighed and observed daily until clinical signs of toxicity subsided, and subsequently every other day throughout the 14-day recovery period.

Findings:

The lowest dosage that produced mortality was 11,000 mg/kg. At this dosage, a 24% body weight loss, lethargy, and limpness were observed. Death occurred 3 days after dosing. At non-lethal dosages, body weight losses of 3-18% occurred within 2 days post dosing. No other clinical signs were observed.

Table B.6.62: Acute oral toxicity of IN-B6895-2

Dose rate (mg/kg)	Dose (mL)	Suspension conc. (mg/mL)	Initial body weight	Mortality ^a
3400	1.5	550	245	0/1
5000	2.3	550	251	0/1
7500	3.4	550	249	0/1
11,000	4.8	550	242	1/1

^a Number of animals that died/number of animals in the dose group

Conclusion:

The oral ALD for IN-B6895-2, a metabolite of bensulfuron methyl, was 11,000 mg/kg of body weight for male rats.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

35) *Approximate lethal dose (ALD) of INN-5297 in rats. Wylie, C.N., Burgess, B., 1983.*

Cross reference IIA, 5.8.1/08

Guidelines:

Not specified

Deviations: Not applicable

GLP:

No

*The study is **acceptable***

Materials and methods:

Test substance: IN-N5297 technical metabolite

Batch: N5297-1

Purity: 95%

IN-N5297-1 was suspended in corn oil and administered by single-dose oral gavage to male CrI:CD[®] rats (1/dose) at doses of 0, 670-11,000 mg/kg. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing.

Findings:

Mortality occurred at dosages of 7,500 and 11,000 mg/kg. At these dosages, lethargy, lacrimation, and laboured breathing occurred. Mortality occurred 2-3 days post-dosing. At non-lethal doses, weight loss, lethargy, wet and/or stained fur, salivation, lacrimation, weakness, ruffled fur, and depressed appetite were observed.

Table B.6.63: Acute oral toxicity of IN-N5297-1

Dose rate (mg/kg)	Dose (mL)	Suspension concentration (mg/mL)	Mortality ^a
670	1.05	150	0/1
1000	1.63	150	0/1
1500	2.37	150	0/1
2250	3.56	150	0/1
3400	2.55	300	0/1
5000	3.70	300	0/1
7500	5.63 ^b	300	1/1
11,000	8.25 ^b	300	1/1

^a Number of animals that died/number of animals in the dose group

^b The dose was administered in 2 portions, 15 minutes apart.

Conclusion:

The oral ALD for IN-N5297-1, a metabolite of bensulfuron methyl, was greater than 7500 mg/kg of body weight for male rats.

36) *Approximate lethal dose (ALD) of INR-9419-1 in rats. Sarver, J., 1985.*

Cross reference IIA, 5.8.1/06

Guidelines:

Not specified

Deviations: Not applicable

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

Toxicology and metabolism

GLP:

No

*The study is acceptable*Materials and methods:

Test substance: IN-R9419 technical metabolite

Batch: R9419-1

Purity: 95%

IN-R9419-1 was suspended in corn oil and administered by single-dose oral gavage to male Crl:CD[®] rats (1/dose) at doses of 0, 5000-11,000 mg/kg. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing.

Findings:

Mortality did not occur at any dosage. At dosages of 7500 and 11,000 mg/kg, diarrhoea was noted in rats.

Table B.6.64: Acute oral toxicity of IN-R9419-1

Dose rate (mg/kg)	Dose (mL)	Suspension concentration (mg/mL)	Mortality ^a
5000	4.2	300	0/1
7500	6.4 ^b	300	0/1
11,000	10.0 ^b	300	0/1

^a Number of animals that died/number of animals in the dose group^b Administered in 2 portions, 15 minutes apartConclusion:

The oral ALD for IN-R9419-1, a metabolite of bensulfuron methyl, was greater than 11,000 mg/kg of body weight for male rats.

37) *Acute oral toxicity study with IN-J290-17 in male and female rats. Finlay, C., 1994.*

Cross reference IIA, 5.8.1./02

Guidelines:

EPA FIFRA Subdivision F, 81-1 (1984); OECD, 401 (1987); EEC 92/69, Annex V-B1 (1992); Japan, 59 NohSan No. 4200 (1985)

Deviations: None

GLP:

Yes

*The study is acceptable*Materials and methods:

Test substance: IN-J290 technical metabolite

Batch: IN-J290-17

Purity: 99.6%

IN-J290-17 in corn oil (50 - 250 mg/mL), was administered by single-dose oral gavage to fasted male and female Crl:CD[®] rats (10/sex) at doses of 1000, 2000, or 2500 mg/kg bw for males and 500, 1000, or 2000 mg/kg bw for females. Rats were dosed at a volume of approximately 10 mL/kg bw. Surviving animals were observed for clinical

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

signs of toxicity, body weight effects, and mortality for up to 14 days after dosing. Rats were examined for gross pathological changes at the end of the 14-day observation period.

Findings:

Clinical signs of toxicity most often observed in male and female rats included lethargy, low posture, low carriage, profuse salivation, ocular discharge, oral discharge and partially closed eyes. Other clinical signs of toxicity included nasal discharge, hunched over posture, moribundity, and diarrhoea. No test substance-related gross lesions were observed at necropsy.

Table B.6.65: Acute oral toxicity of IN-J290-17

Males				Females			
Doses (mg/kg)	Mortality ^a	Time of death	LD ₅₀ mg/kg	Doses (mg/kg)	Mortality ^a	Time of death	LD ₅₀ mg/kg
1000	3/10	D-1 & 2	1510	500	1/10	D-2	920
2000	5/10	D-1 & 2	(670-2200) ^b	1000	5/10	D2 & 3	(680-1240) ^b
2500	9/10	D-1 – D-3		2000	10/10	D-1 & 2	

^a Number of animals which died/number of animals in dose group

^b 95% Confidence interval in parenthesis

^c D - Test-day following dosing.

Conclusion:

The combined oral LD₅₀ for IN-J290-17, a metabolite of bensulfuron methyl, in male and female rats was 1200 mg/kg bw.

38) *Ten-dose oral subacute study in rats. Wylie, C.N., 1983.*

Cross reference IIA, 5.8.1./09

Guidelines:

Not specified.

Deviations: A range-finding study that differs from EEC 92/69, Annex V, Method B.7 (1992) as follows: 6 male rats/dose; single dose group, 450 mg/kg bw/day for 5 days/week for 2 weeks *via* gavage; gross and histopathological exams on 3 rats each after dosing and 14-days recovery.

GLP:

No

The study is considered as Additional Data

Materials and Methods:

Test substance: IN-J290-1 technical metabolite

Batch: IN-J290-1

Purity: 95%

A ten-dose oral subchronic test was performed with IN-J290-1. The test substance, as a suspension in corn oil, was administered by intragastric intubation to six male CrI:CD[®]BR rats. A concurrent control group was intubated with corn oil. Rats were dosed 10 times over a 2-week period (weekends excluded) at a dose rate of 450 mg/kg bw/day. Rats were observed daily throughout the dosing period (weekends excluded). At the end of the dosing period, 3 rats

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Toxicology and metabolism

from each group were sacrificed for pathological examination. The remaining rats were observed for 14 days after cessation of dosing and were then sacrificed for pathological examination.

Selected tissues were weighed and the following tissues were examined microscopically: liver, kidneys, urinary bladder, lungs, heart, spleen, thymus, pancreas, adrenal glands, thyroid gland, trachea, oesophagus, brain, duodenum, jejunum, ileum, cecum, colon, mesenteric and mediastinal lymph nodes, testes, epididymides, sternum, bone marrow, eyes, and gross lesions.

Findings:

No deaths occurred during the study. Clinical signs observed during the test period were salivation and moderate to severe initial weight loss followed by recovery. No gross compound-related effects were noted in rats sacrificed after the 10th dose or after the 2-week recovery period. All microscopic findings were believed to be incidental or spontaneous and were not considered the result of compound administration.

Conclusion:

In this ten-dose oral subacute test with IN-J290-1, the no-observed-adverse-effect level (NOAEL) was greater than 450 mg/kg bw/day for male rats.

***In vitro* gene mutation - bacteria**

39) *Mutagenicity testing of IN-J290-17 in the Salmonella typhimurium and Echerichia coli plate incorporation assay, Revision No. 2. Bentley, K.S., 1994*

Cross reference IIA, 5.8.1./01

Guidelines:

EEC Council Directive 67/548/EEC, Methods B.13 and B.14; OECD Guidelines for Testing of Chemicals, No. 471; U.S. EPA Pesticide Assessment Guidelines, Subdivision F 84-2; and Japan MAFF, 59 NohSan No. 4200

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: IN-J290-17 technical metabolite

Batch: J290-17

Purity: 99.6%

IN-J290-17 was evaluated for mutagenicity in *Salmonella typhimurium* strains TA1535, TA97, TA98, and TA100 and *E. coli* strain WP2uvrA(pKM101) with and without an exogenous metabolic activation system, S9 fraction mix. The S9 fraction mix contained the following: 8 mM MgCl₂, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP⁺, 100 mM sodium phosphate pH 7.4, and 1.6 mg S9 fraction/1.0 mL S9 fraction mix. The S9 fraction was the 9000 x g supernatant of liver homogenate (1 g liver; 3.0 mL KCl). Livers were obtained from 8 – 9 week old male Sprague-Dawley rats injected i.p. with Arochlor® 1254 @ 500 mg/kg bw.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

Two independent trials with and without metabolic activation were conducted. Concentrations of 10, 50, 100, 500, 1000, 2500, and 5000 µg IN-J290-17/plate were evaluated using standard plate incorporation methods. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). The positive indicator for trials with metabolic activation was 2-aminoanthracene. Positive indicators for trials without metabolic activation were: 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), ICR-191 Acridine (TA97), and methyl methane sulfonate [WP2_{uvr}A(pKM101)]. Deionised water was the solvent for sodium azide, ICR-191 Acridine, and methyl methane sulfonate. Other positive indicators were dissolved in DMSO.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation (). Plates treated with the positive indicators had significantly increased numbers of revertant colonies per plate when compared to the solvent controls.

Table B.6.66: IN-J290-17: Summary of mean revertants/plate

Mean ^a revertants/plate without activation											
Compound	Conc. µg/plate	TA100		TA1535		TA97		TA98		WP2 _{uvr} (pKM101)	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial II	Trial II
DMSO	0	141	98	19	14	138	146	15	25	154	167
IN-J290-17	10	134	93	14	9	126	135	15	18	138	168
	50	137	109	11	11	149	159	12	22	142	152
	100	143	109	14	7	152	126	12	21	140	163
	500	154	93	12	13	150	127	13	19	145	169
	1000	137	118	14	7	160	138	17	22	160	167
	2500	145	114	9	10	126	184	14	25	147	159
	5000	133	94	12	3	113	154	14	17	121	106
NAAZ ^b	2	549	809	324	173	ne	ne	ne	ne	ne	ne
2NF	25					ne	ne	554	1657	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	1156	721
ICR-191	2	ne	ne	ne	ne	769	678	ne	ne	ne	ne
Mean ^a revertants/plate with activation											
DMSO	0	149	109	43	21	161	170	21	23	102	182
IN-J290-17	10	135	105	42	22	141	165	17	20	96	182
	50	152	103	50	20	145	119	17	26	96	178
	100	147	108	50	20	145	156	19	25	95	152
	500	157	99	42	18	144	117	21	26	104	177
	1000	138	109	53	13	139	208	18	24	103	168
	2500	151	106	35	20	131	119	15	27	88	168
	5000	147	86	26	15	134	156	17	18	68	145
2AA	1	874	520	ne	ne	1156	732	ne	ne	ne	ne
	2	ne	ne	587	260			880	2257	ne	ne
	25	ne	ne	ne	ne	ne	ne	ne	ne	693	878

^a Average 3 replicates per trial

^b 2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; MMS = methyl methane sulfonate; NAAZ = sodium azide;

ICR-191 – ICR-191 Acridine; DMSO = dimethyl sulfoxide

ne = Not evaluated

Conclusion:

IN-J290-17, a metabolite of bensulfuron methyl, was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

40) *Mutagenicity evaluation of INB-6895-2 in Salmonella typhimurium (Ames assay). Rickard, L.B., 1985a*

Cross reference IIA, 5.8.1./04

Guidelines:

Not stated

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-B6895 technical metabolite

Batch: B6895-2

Purity: 99.4%

IN-B6895-2 was evaluated for mutagenicity in *Salmonella typhimurium* strains TA1535, TA97, TA98, and TA100 with and without an exogenous metabolic activation system (Aroclor® 1254-induced rat liver S9 (10% w/v), 1.6 mg/mL protein). Nominal concentrations of 10, 50, 100, 250, 500 µg/plate (without activation) and 50, 100, 500, 100, 2500 µg/plate (with activation) were evaluated in 2 trials using standard plate incorporation methods. The highest dose level was set based on cytotoxicity and preliminary mutagenicity testing in the presence and absence of an activation system, as measured in strain TA98. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). Positive indicators were 2-aminoanthracene, 9-aminoacridine, N-methyl-N-nitro-N-nitrosoguanidine, and 2-nitrofluorene. A test substance was classified as positive when the number of induced revertants at one or more of the test sample concentrations studied were at least two times greater than the number of revertants in the solvent control. These dose levels must have a probability was less than 0.01 that the number of induced revertants are the same as the spontaneous revertant number, and the probability was less than 0.01 that there was not a positive correlation between the number of revertants and increasing concentrations of the test samples.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation (). Plates treated with the positive indicators had significantly increased numbers of revertant colonies per plate when compared to the solvent controls. No significant increases in revertants or positive linear dose-responses were obtained in any of the strains. There was no evidence of toxicity by the test compound.

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

Toxicology and metabolism

Table B.6.67: IN-B6895: Summary of mean revertants/plate

Mean ^a revertants/plate without activation									
Compound	Conc. µg/plate	TA1535		TA97		TA98		TA100	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
DMSO	0	25.5	20.0	124.5	84.5	19.5	17.0	81.5	116.0
IN-B6895-1	10	24.5	20.5	118.0	87.5	17.5	14.5	81.5	111.0
	50	25.0	21.0	105.5	90.5	19.5	17.5	111.5	117.0
	100	21.5	25.5	115.0	87.0	16.5	17.5	119.0	120.0
	250	19.0	23.5	99.0	86.0	13.0	16.5	129.5	102.0
	500	16.0	25.5	99.5	75.5	17.5	15.0	106.0	107.0
9Aac ^b	50	ne	ne	522.5	617.0	ne	ne	ne	ne
2NF	25	ne	ne	ne	ne	1700.5	1873.0	ne	ne
MNNG	4	2558	2606	ne	ne	ne	ne	3114.0	2582.0
Mean ^a revertants/plate with activation									
DMSO	0	16.0	17.5	124.0	115.5	30.5	27.5	117.5	110.5
IN-B6895-1	50	12.5	13.5	119.5	119.5	33.5	22.0	110.0	109.5
	100	12.0	18.5	115.5	112.5	30.0	28.0	103.0	110.5
	500	14.0	9.5	128.0	110.5	34.5	28.0	111.0	111.5
	1000	20.0	14.0	120.0	111.5	33.5	29.5	89.5	103.5
	2500	14.0	16.5	85.0	113.5	25.0	29.0	92.0	95.5
2AA	1	ne	ne	1458	940.5	ne	ne	2950.5	1537.5
	2	214	149.5	ne	ne	2176.0	2692.5	ne	ne

^a Average 2 replicates per trial^b 9Aac = 9-aminoacridine; 2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; DMSO = dimethyl sulfoxide
ne = Not evaluatedConclusion:

IN-B6895-2, a metabolite of bensulfuron methyl, was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

41) *Mutagenicity evaluation of INR-9419-1 in Salmonella typhimurium. Rickard, L.B., 1985b.*

Cross reference IIA, 5.8.1./05

Guidelines:

Not stated

GLP:

Yes

The study is acceptable

Materials and Methods

Test Substance: IN-R9419 technical metabolite

Batch: R9419-1

Purity: 95%

IN-R9419-1 was evaluated for mutagenicity in *Salmonella typhimurium* strains TA1535, TA97, TA98, and TA100 with and without an exogenous metabolic activation system (Aroclor[®]1254-induced rat liver S9 (10% w/v), 1.6 mg/mL protein). Nominal concentrations of 50, 500, 1000, and 5000 µg/plate were evaluated in 2 trials using

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

Toxicology and metabolism

standard plate incorporation methods. A third trial was performed in strain TA98 without activation due to the abnormally low number of revertants/plate for the positive indicator in Trial I. Only data from strain TA98 Trials II and III without activation were used in the statistical analysis. The highest dose level was set based on cytotoxicity and preliminary mutagenicity testing in the presence and absence of an activation system, as measured in strain TA98. The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). Positive indicators were 2-aminoanthracene, 9-aminoacridine, N-methyl-N'-nitro-N-nitrosoguanidine, and 2-nitrofluorene. A test substance was classified as positive when the number of induced revertants at one or more of the test sample concentrations studied were at least two times greater than the number of revertants in the solvent control. These dose levels must have a probability less than 0.01 that the number of induced revertants are the same as the spontaneous revertant number, and the probability was less than 0.01 that there was not a positive correlation between the number of revertants and increasing concentrations of the test samples.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation (). No significant increases in revertants or positive linear dose-responses were obtained in any of the strains. Slight cytotoxicity was observed on higher test substance concentrations.

Table B.6.68: IN-R9419: Summary of mean revertants/plate

Mean ^a revertants/plate without activation										
Compound	Conc. µg/pl ate	TA1535		TA97		TA98			TA100	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial III	Trial I	Trial II
DMSO	0	21.5	36.0	86.0	81.0	16.5	19.0	20.0	92.0	133.0
IN-R9419-1	50	22.0	30.0	71.5	77.5	11.5	19.5	22.0	101.0	135.0
	100	17.5	31.0	68.5	82.0	12.5	13.5	21.5	87.0	114.0
	500	23.0	31.0	83.0	90.5	13.0	12.5	13.0	78.5	134.5
	1000	19.0	35.0	78.5	81.5	10.5	18.5	8.5	91.5	132.0
	5000	12.5	20.0	72.0	84.0	18.5	18.0	23.5	88.0	128.5
9Aac ^b	50	ne	ne	614.0	342.0	ne	ne	ne	ne	ne
2NF	25	ne	ne	ne	ne	54.5	2154.5	1989.0	ne	ne
MNNG	4	3119	3223.5	ne	ne	ne	ne	ne	2738.5	3012.0
Mean ^a revertants/plate with activation										
DMSO	0	10.5	14.5	97.0	121.5	30.5	30.0	ne	101.5	116.5
IN-R9419-1	50	14.0	19.5	93.5	103.5	28.0	29.5	ne	100.5	113.0
	100	10.0	17.0	103.5	92.0	24.0	29.5	ne	94.5	119.5
	500	13.5	13.5	97.0	106.0	32.0	30.5	ne	98.0	119.0
	1000	12.5	12.5	96.0	94.5	31.0	32.5	ne	103.5	121.5
	5000	13.5	12.0	107.5	88.5	28.5	33.0	ne	107.0	109.5
2AA	1	ne	ne	849.5	833.0	ne	ne	ne	2015.5	1832.0
	2	165.5	215.5	ne	ne	2824.0	2916.5	ne	ne	ne

^a Average 2 replicates per trial

^b 9 Aac = 9-aminoacridine; 2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; DMSO = dimethyl sulfoxide

ne = Not evaluated

Conclusion:

IN-R9419-1, a metabolite of bensulfuron methyl, was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

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

Toxicology and metabolism

42) *Mutagenicity evaluation in Salmonella typhimurium. Summers, J.C., 1983.*

Cross reference IIA, 5.8.1./07

Guidelines:

Not stated

GLP:

Not specified

The study is acceptable

Materials and Methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-1

Purity: 95%

IN-N5297-1 was evaluated for mutagenicity in *Salmonella typhimurium* strains TA1535, TA97, TA98, and TA100 with and without an exogenous metabolic activation system (Aroclor[®] 1254-induced rat liver S9 (10% w/v), 1.6 mg/mL protein). Nominal concentrations of 10, 50, 100, 500, 1000, or 5000 µg/plate (without activation) and 50, 100, 500, 1000, 5000, and 10,000 µg/plate (with activation) were evaluated in 2 trials using standard plate incorporation methods. The highest dose level was set based on cytotoxicity and preliminary mutagenicity testing in the presence and absence of an activation system, as measured in strain TA1535 (data not reported in the original study). The test substance was administered to the test system as a solution in dimethyl sulfoxide (DMSO). Positive indicators were 2-aminoanthracene, 9-aminoacridine, N-methyl-N'-nitro-N-nitrosoguanidine, and 2-nitrofluorene. A test substance was classified as positive when the probability was less than 0.01 that the numbers of revertants at one or more of the test sample concentrations studied were not greater than the number of revertants in the solvent control, and the probability was less than 0.01 that there was not a positive correlation between the number of revertants and increasing concentrations of the test samples.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation. There was no evidence of toxicity by the test compound.

WARNING: This document forms part of an EC evaluation data package and should not be used 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

Toxicology and metabolism

Table B.6.69: IN-N5297: Summary of mean revertants/plate

Mean ^a revertants/plate without activation									
Compound	Conc. µg/plate	TA1535		TA97		TA98		TA100	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
DMSO	0	14.6	14.0	98.7	106.7	17.7	13.3	96.3	99.3
IN-N5297-1	10	18.0	19.0	85.5	95.5	15.5	12.5	113.0	100.5
	50	22.0	13.5	106.0	95.5	17.0	14.5	86.0	89.0
	100	19.5	11.5	93.5	95.5	12.0	13.0	98.5	96.5
	500	19.5	16.5	101.5	104.5	19.0	10.5	110.0	81.0
	1000	19.0	10.5	105.5	102.0	21.5	14.0	111.5	97.0
	5000	ne	14.0	ne	89.0	ne	14.0	ne	89.0
9Aac ^b	50	ne	ne	810.5	677.0	ne	Ne	ne	ne
2NF	25	ne	ne	ne	ne	2430.5	2308.5	ne	ne
MNNG	4	2388.5	2382.5	ne	ne	ne	ne	3605.5	3347.0
Mean ^a revertants/plate with activation									
DMSO	0	11.6	10.0	117.7	113.3	36.7	24.7	100.7	88.7
IN-N5297-1	50	14.5	10.5	105.5	112.0	40.0	22.5	104.0	97.0
	100	11.5	10.0	116.0	108.0	35.5	27.0	100.0	103.5
	500	9.5	9.0	116.5	109.0	29.5	22.0	105.0	89.0
	1000	10.5	9.0	96.5	116.5	35.0	24.5	100.5	86.0
	5000	14.0	11.5	105.5	99.0	36.5	20.0	112.0	92.0
	10,000	ne	10.0	ne	52.5	ne	21.0	ne	93.5
2AA	1	ne	ne	2275.0	2227.0	ne	ne	1322.0	1084.5
	2	142.5	160.5	ne	ne	2500.5	1905.5	ne	ne

^a Average 2 replicates per trial^b 9Aac = 9-aminoacridine; 2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; DMSO = dimethyl sulfoxide
ne = Not evaluated

Conclusion:

- 43) *IN-N5297-1, a metabolite of bensulfuron methyl, was negative for mutagenic activity in the non-activated and S9-activated test systems in the in vitro bacterial gene mutation assay.*

Summary of studies on bensulfuron methyl metabolites

Supplementary studies were conducted on four bensulfuron methyl metabolites. The studies primarily consisted of acute oral toxicity range-finding studies and evaluations in a bacterial mutagenicity assay (Ames test). The metabolites represented those resulting from the following: o-demethylation of the phenyl ring (IN-R9419); hydrolysis of the sulfonylurea bridge to release the pyrimidine component (IN-J290) and the phenyl sulfonamide (IN-N5297); and rearrangement of the phenyl sulfonamide to the homosaccharin (IN-B6895) [Figure B.6. 1]. Based on this limited testing, none of the metabolites were mutagenic and none produced significant toxicity when administered by oral gavage. Only IN-J290 would be classified under Directive 67/548/EEC as harmful for acute oral toxicity (LD₅₀ 1200 mg/kg bw). However, its presence as a trace component resulting from metabolism of very low levels of bensulfuron methyl likely associated with human exposure does not represent a risk to human health.

RMS considers the metabolite IN-J290 as not relevant with regard to mammalian toxicology, in light of findings from toxicity studies on metabolite itself, toxicity studies on bensulfuron methyl and the position paper submitted by the notifier.

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Italy	May 2006	Bensulfuron methyl

Toxicology and metabolism

B.6.8.2 Supplementary studies on the active substance

B.6.8.2.1 Acute neurotoxicity study – rats

Bensulfuron methyl was evaluated for its potential to produce neurotoxicity in laboratory animals administered sub lethal doses. For this evaluation, existing acute, subchronic, reproduction, developmental, and chronic toxicity studies in rodents and non-rodents were considered. Clinical observations indicative of potential effects on the central and peripheral nervous system were evaluated, and no observations suggestive of neurotoxicity were noted. This evaluation was further supported by the absence of gross and microscopic effects on nervous system tissues.

Bensulfuron methyl is not structurally related to a known neurotoxin or class of neurotoxins. It is an herbicide; and therefore, its pesticide mode of action is designed to interact with plants, not the mammalian nervous system. Therefore, an acute neurotoxicity study was not required for bensulfuron methyl.

B.6.8.2.2 Subchronic neurotoxicity study – rats

Bensulfuron methyl was evaluated for its potential to produce neurotoxicity in laboratory animals administered sub lethal doses. For this evaluation, existing acute, subchronic, reproduction, developmental, and chronic toxicity studies in rodents and non-rodents were considered. Clinical observations indicative of potential effects on the central and peripheral nervous system were evaluated, and no observations suggestive of neurotoxicity were noted. This evaluation was further supported by the absence of gross and microscopic effects on nervous system tissues.

Bensulfuron methyl is not structurally related to a known neurotoxin or class of neurotoxins. It is an herbicide; and therefore, its pesticide mode of action is designed to interact with plants, not the mammalian nervous system. Therefore, a subchronic neurotoxicity study was not required for bensulfuron methyl.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Medical surveillance on manufacturing plant personnel

44) *Medical exam and summaries of employees manufacturing and formulating DuPont sulfonylurea herbicides: Manati, Puerto Rico facility. O'Neal, F.O., 1996.*

Cross reference IIA, 5.9.1.01

Guideline:

Not applicable

Deviations: Not applicable

GLP:

Not applicable

Material and methods

Test Substance: DPX-F5384

Batch: Not applicable

Purity: Not applicable

DuPont has produced bensulfuron methyl on a commercial scale for more than 17 years. The Manati, Puerto Rico manufacturing site has produced bensulfuron methyl for the vast majority of this time. The medical records of 30

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randomly selected employees, who work at Manati in the manufacturing, formulation, or packaging areas for bensulfuron methyl were reviewed. An evaluation of these records indicated the parameters measured (including clinical biochemistry, urinalysis, haematology, chest X-rays, and electrocardiograms–EKGs) were within the normal published ranges or were normal with respect to the opinion of the examining physician. Therefore, there were no occupational illnesses or symptoms associated with this product.

B.6.9.2 Direct observation, e.g., clinical cases and poisoning incidents

A search covering the time period from 1994 to the present was conducted on available reports and documentation from the open literature relating to clinical cases and poisoning incidents related to the agricultural use of bensulfuron methyl and mixtures of bensulfuron methyl with propanil and metsulfuron methyl.

There have been no reported accidental poisonings with bensulfuron methyl and mixtures.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

A search was undertaken on 19 April 2004, on available reports and documentation from the open literature relating to clinical cases and poisoning incidents. The search found no relevant hits in regards to the key words listed.

B.6.9.4 Diagnosis of poisoning

No specific human or animal symptoms of bensulfuron methyl acute or subacute toxicity are known.

B.6.9.5 .Proposed treatment: first-aid measures, antidotes, medical treatment

There is no specific antidote. Treat symptomatically.

- Inhalation.* Move to fresh air. Oxygen or artificial respiration if needed. Consult a physician after significant exposure.
- Skin contact.* Wash off immediately with soap and plenty of water.
- Eye contact.* Rinse immediately with plenty of water, also under the eyelids. Keep eye wide open while rinsing. If eye irritation persists, consult a specialist.
- Ingestion.* Call a physician immediately. Drink 1 or 2 glasses of water. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person.

Note to physician: Efforts should be directed toward alleviation of any symptoms of illness and to prevent further absorption of bensulfuron methyl.

B.6.9.6 Expected effects of poisoning

There are no specific known human effects of exposure to bensulfuron methyl. Therefore, no specific intervention is indicated (except to prevent further absorption) and the patient should be treated symptomatically. No symptoms would be expected to result from dermal exposure. Exposure to dust or vapours of bensulfuron methyl could cause irritation of the eyes and the respiratory tract.

Prolonged overexposure could result in body weight loss, reductions in red blood cell parameters, liver enlargement, and increases in hepatic enzyme activities. In dogs, microscopic changes in the liver, including bile stasis, centrilobular hepatocellular swelling, and individual hepatocyte necrosis, were observed at the highest concentration tested, 10,000 ppm. Adverse effects on organs were not detected in other species tested.

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Toxicology and metabolism

B.6.9.7 Summary of medical data

DuPont has produced bensulfuron methyl on a commercial scale for more than 17 years. The Manati, Puerto Rico manufacturing site has produced bensulfuron methyl for the vast majority of this time. The medical records of randomly selected employees, who work at Manati in the manufacturing, formulation or packaging areas for bensulfuron methyl were reviewed. An evaluation of these records indicated the parameters measured were within the normal published ranges or were normal with respect to the opinion of the examining physician. Therefore, there were no occupational illnesses or symptoms associated with this product.

There have been no reported accidental poisonings with bensulfuron methyl and there are no known specific human effects. Therefore, no specific intervention or antidote is indicated. In case of poisoning, the patient should be treated symptomatically.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of mammalian toxicology

Metabolism

At doses relevant to human exposure, bensulfuron methyl was rapidly and extensively absorbed from the gastrointestinal tract (e.g., >83 and >90% absorbed for female and male rats, respectively). It was extensively metabolised in rats; primarily by *O*-demethylation and hydroxylation of the pyrimidine ring as well as sulfonamide bond cleavage. Although urine was the predominant route of elimination, significant amounts were also eliminated through the bile and to a lesser extent through the faeces. Elimination was rapid, with plasma elimination half-lives ranging from approximately 5- to 9-hours. Excretion in the urine, bile, and faeces was essentially complete within 48- to 72-hours. Tissue clearance was also rapid and there was no evidence of accumulation, e.g., ≤0.3% of ¹⁴C-residues remained in tissues after 96 hours.

Acute toxicity

Results of acute toxicity studies in rats, rabbits, and guinea pigs indicate that bensulfuron methyl has no significant acute toxicity via the oral, dermal, and inhalation routes of exposure. Bensulfuron methyl is not an eye or skin irritant and does not cause skin sensitisation.

Subchronic toxicity

In subchronic (90-day) feeding study in rats, the NOAEL was 1500 ppm (93 mg/kg/day) in males and 7500 ppm (567 mg/kg/day) in females based on mild haematology effects in males. The haematology effects typically consisted of decreases in number of erythrocytes, haemoglobin concentration, and haematocrit.

The NOAEL in the 90-day study with bensulfuron methyl in dogs was 1000 ppm (32 mg/kg/day in males and 37 mg/kg/day in females) based on elevations in the activities of alkaline phosphatase and alanine aminotransferase that were consistent with gross and microscopic liver changes at the 10,000 ppm dietary concentration. Body weight and food efficiency were also lower in 10,000 ppm males and females. However, in the one-year study in dogs, the NOEL was 750 ppm (21.4 and 19.9 mg/kg/day for males and females, respectively) based on liver enlargement.

Mice were less sensitive to the subchronic toxicity of bensulfuron methyl relative to the rat and dog, with NOAELs of 1272 and 1401 mg/kg/day in males and females, respectively. These NOAELs were based on the absence of adverse effects at dietary concentrations up to 10,000 ppm.

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Toxicology and metabolism

Chronic toxicity

Chronic studies with bensulfuron methyl were conducted in rats and mice to determine the carcinogenic potential and/or chronic toxicity of the compound. Effects generally similar to those seen in the 90-day studies were seen in the chronic studies. Bensulfuron methyl was not carcinogenic in the rat or mouse.

The chronic NOAEL in rats was 750 ppm for both males and females, equivalent to 30 and 40 mg/kg/day, respectively. In male rats, the NOAEL was based on body weight, nutritional effects, decreased haemoglobin, and histological changes in the liver at 7500 ppm. In females, the NOAEL was based on body weight and nutritional effects, and histological changes in the liver at 7500 ppm. The chronic NOAEL in mice was 5000 ppm for both males and females, equivalent to 455 and 460 mg/kg/day, respectively, based on the absence of persistent, adverse effects at any dietary concentration in either males or females. The NOAEL in both male and female dogs fed bensulfuron methyl for 1-year was 750 ppm (21.4 mg/kg/day in males and 19.9 mg/kg/day in females) based on increases in alanine aminotransferase activity, alkaline phosphatase activity, liver weight, and liver to body weight ratio at 7500 ppm.

Genetic toxicity

Most genetic toxicity studies, including a rat *in vivo* bone marrow study, were negative. However, bensulfuron methyl was positive in an *in vitro* Sister Chromatid Exchange assay without metabolic activation. However, the sister chromatid exchange assay is considered to be less than ideal for detecting effects due directly to a chemical (Preston, R.J. "Chromosomal Changes" in The Use of Short- and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation. Ed. by D.B. McGregor, J.M. Rice, and S. Venitt, IARC Scientific Publications No. 146.) Based on these studies, the weight of evidence indicates that bensulfuron methyl is not genotoxic.

Reproductive and developmental toxicity

In the two-generation reproduction toxicity study with bensulfuron methyl the NOAEL for parental toxicity was 250 ppm (19.5–22.3 mg/kg bw/day) based on slight reductions in body weight in F₁ adult males at 7500 ppm and above. The NOAEL for reproductive and fertility effects was 20,000 ppm (1404.5–1874.0 mg/kg bw/day), based on the absence of any effects at any dietary concentration. The NOAEL for effects on pup growth and development was 250 ppm (22.3 mg/kg bw/day for F₁ dams), based on a reduction in the body weight of F₁ pups at 7500 ppm and above. Developmental toxicity studies were conducted with bensulfuron methyl in rats and rabbits. Bensulfuron methyl was not teratogenic. In the developmental toxicity study in rats, the NOAEL for maternal effects was ≥2000 mg/kg/day based on the absence of maternal toxicity at 2000 mg/kg bw/day. The foetal NOAEL was 500 mg/kg/day, based on the adverse effects on pup weight, and the presence of variations in pups at 2000 mg/kg/day.. In the developmental study conducted in rabbits, both the maternal and foetal NOAEL was 300 mg/kg/day based on death of females dosed with 1500 mg/kg/day and on the decreased maternal body weight and food consumption, and on total resorptions and decreased pup body weight at 1500 mg/kg bw/day.

Neurotoxicity

Bensulfuron methyl was evaluated for its potential to produce neurotoxicity in laboratory animals administered sub lethal doses. For this evaluation, existing acute, subchronic, reproduction, developmental, and chronic toxicity studies in rodents and non-rodents were considered. Clinical observations indicative of potential effects on the central and peripheral nervous system were evaluated, and no observations suggestive of neurotoxicity were noted. This evaluation was further supported by the absence of gross and microscopic effects on nervous system tissues.

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

Toxicology and metabolism

Bensulfuron methyl is not structurally related to a known neurotoxin or class of neurotoxins. It is an herbicide, and therefore, its pesticide mode of action is designed to interact with plants, not the mammalian nervous system.

B.6.10.2 Conclusions - mammalian toxicology

Bensulfuron methyl has no significant acute toxicity *via* the oral, dermal, and inhalation routes of exposure. It is not an eye or skin irritant and does not cause skin sensitisation. Bensulfuron methyl is not genotoxic, carcinogenic, or teratogenic.. In the developmental toxicity study in rats, the NOAEL for maternal effects was ≥ 2000 mg/kg/day based on the absence of maternal toxicity at 2000 mg/kg bw/day. The foetal NOAEL was 500 mg/kg/day, based on the adverse effects on pup weight, and the presence of variations in pups at 2000 mg/kg/day. In the developmental study conducted in rabbits, both the maternal and foetal NOAEL was 300 mg/kg/day based on death of females dosed with 1500 mg/kg/day and on the decreased maternal body weight and food consumption, and on total resorptions and decreased pup body weight at 1500 mg/kg bw/day. The lowest effect levels following exposure to bensulfuron methyl are based on increases in alanine aminotransferase activity, alkaline phosphatase activity, liver weight, and liver to body weight ratio at 7500 ppm in male and female dogs.

The lowest NOAEL is 19.9 mg/kg/day, based on clinical chemistry and organ weight changes in the 1-year study in dogs.

B.6.10.3 Acceptable Operator Exposure Level (AOEL)

Results of the bensulfuron methyl 90-day feeding studies, 1-year dog study, and the developmental and reproductive toxicity studies are considered appropriate for determination of the AOEL. Table B.6. 74 summarises the NOAELs and LOAELs observed in those studies, according to the EU document 7531/VI/95-rev 6 adopted by the Scientific Committee on Plants on 23 October 2002, "Guidance for the setting of AOELs".

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

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Toxicology and metabolism

Table B.6. 74: AOEL derivation: Summary of relevant short-term toxicity studies with bensulfuron methyl

Study/Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at the LOAEL	Reference
90-day rat^a M: 0, 6, 93 and 475 F: 0, 7, 111, and 567	93 (M ^b) 567 (F ^b)	475 (M) n.d. (F)	Changes in haematology parameters	O'Neal, 1986
90-day mouse M: 0, 38.9, 132 and 1272 F: 0, 39.2, 133, and 1401	1272 (M) 1401 (F)	n.d. (M) n.d. (F)		Hirano, 1984
90-day dog M: 0, 3.3, 32 and 341 F: 0, 3.5, 37 and 359	32 (M) 37 (F)	341 (M) 359 (F)	Clinical chemistry changes. Gross and microscopic findings in liver.	Stadler, 1986
1-year dog M: 0, 1.4, 21.4 and 237.3 F: 0, 1.4, 19.9 and 222.6	21.7 (M) 19.9 (F)	237.3 (M) 222.6 (F)	Liver weight increased. Changes in clinical chemistry (ALT and ALP).	Bolte, 1986
Developmental rat 0, 50, 500 and 2000 By corn oil gavage	Maternal: 2000 Foetal: 500	Maternal: n.d. Foetal: n.d.	Pup weight Altered ossification	Lamontia, 1999
Developmental rabbit 0, 30, 300 and 1500 By methyl cellulose gavage	Maternal: 300 Foetal: 300	Maternal: 1500 Foetal: 1500	Maternal: Signs of toxicity and deaths. Foetal: Mean foetal body weight decreases. Increases in total resorptions.	Zellers, 1985
Two generation reproduction, rat 0, 250, 7500, and 20000 ppm in the diet	Parental: 250 ppm (19.5 mg/kg bw/day F ₁ M) Repro/Fertility: 20000 ppm (M-1201.0 – 1589.5 mg/kg bw/day, F-1404.4-1874.0 mg/kg bw/day) Pup: 250 ppm (22.3 mg/kg bw/day F ₁ Dams)	Parental: 7500 ppm Repro/Fertility: n.d. Pup: 7500 ppm	Parental: Decreases in body weight Pup: Body weight decreases	Mullin, 1989

^a Not determined^b M = Male, F = Female

The 1-year dog study has the lowest relevant NOAEL (19.9 mg/kg bw/day) for establishing the AOEL. The dog's response to bensulfuron methyl administration results in more significant systemic toxicity, e.g., effects on liver enzymes and liver histopathology. The 1-year dog study NOAEL was based on clinical chemistry and organ weight effects indicative of liver toxicity.

Even if in developmental study in rat there is a foetal effect (reduced pup weight and abnormal ossification) at doses lower than those eliciting parental toxicity, the NOAEL is higher (22 fold) than the 1-year dog study NOAEL; furthermore in the rat reproduction study the parental systemic toxicity NOAEL is pretty near (19.5 mg/kg bw/day). That's why choosing the 1-year dog study NOAEL will prevent embryotoxic effects, which may be induced by a single exposure during the critical phase.

Using the NOAEL of 19.9 mg/kg bw/day and applying a default safety factor of 100 results in an AOEL of 0.2 mg/kg/day (absorbed dose). The metabolism study conducted in rats indicates that bensulfuron methyl is almost

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completely absorbed from the gastrointestinal tract, thus no correction for oral absorption is applied. The proposed AOEL is 0.2 mg/kg bw/day.

B.6.10.4 Acceptable Daily Intake (ADI)

The chronic studies in rats and mice and 1-year dog study were considered the appropriate as a basis for the ADI. Of these three studies the lowest NOAEL was observed in the 1-year dog study. This NOAEL was based on liver weight increases and changes in clinical parameters (ALT and ALP). Although the 90-day dog study provides a higher NOAEL due to dose spacing and effects at the LOAEL are similar, the 1-year dog study was selected as a more conservative estimate. Therefore, the proposed ADI is 0.2 mg/kg bw/day, based on the NOAEL of 19.9 mg/kg bw/day applying a default 100 fold safety factor.

Table B.6.75: ADI derivation: Summary of bensulfuron methyl long-term toxicity studies

Study/doses (mg/kg bw /day)	NOAEL (mg/kg bw /day)	LOAEL (mg/kg bw /day)	Effects at the LOAEL	Reference
2-Year Rat M: 2, 30, 309 F: 2.7, 40, 405	30 (M) ^a 40 (F)	309 (M) 405 (F)	Reduced body weights Reduced red blood cell mass Liver histopathological changes	Stadler, 1986
2-Year Mouse M: 0.87, 13.4, 226, 455 F: 0.93, 13.6, 227, 460	455 (M) 460 (F)	>455(M) >460 (F)	No adverse effects at the highest dose tested.	Maita, 1985
1-Year Dog M: 1.4, 21.4, 237.3 F: 1.4, 19.9, 222.6	21.7 (M) 19.9 (F)	237.3 (M) 222.6 (F)	Increased liver weight. Increased liver leakage enzymes (ALT, ALP)	Bolte, 1986

^a M = Male, F = Female

B.6.10.5 Parametric value for drinking water

The WHO approach to the Maximum Allowable Concentration (MAC) in water is to allocate one tenth of the ADI to water and this allocation is converted to a concentration in water (*i.e.*, MAC) as follows:

$$\frac{\text{ADI} \times \text{body weight} \times \text{P}}{\text{C}}$$

Where:

- Body weight = body weight (60 kg) for adults
P = fraction of the ADI allocated to drinking water (*i.e.*, 1/10)
C = daily consumption of water (2 litres)

Therefore the MAC for bensulfuron is 0.6 mg/L

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

Toxicology and metabolism

B.6.11 Acute toxicity including irritancy and skin sensitization of preparations (Annex IIIA 7.1)

B.6.11.1 Acute toxicity - oral

45) *Acute oral toxicity study with IN-F5384-85 in rats. Sarver, J.W., 1986.*

Cross reference IIIA, 7.1.1./01

Guidelines:

USEPA 81-1

Deviations: None

GLP:

Yes

*The study is **acceptable***

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.s./kg

Bensulfuron methyl 60WG in distilled water, was administered by single-dose oral gavage to fasted male and female Crl:CD®BR rats (5/sex) at a dose of 5000 mg/kg. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing. At the end of the 14-day observation period, rats were examined for gross pathological changes.

Findings:

No mortalities were observed (Table B.6.76). No clinical signs of toxicity were observed during the study. Test substance-related slight body weight loss (1-4%) was noted in female rats only. No test substance-related gross lesions were observed at necropsy.

Table B.6.76: Acute oral toxicity of Bensulfuron methyl 60WG

Dose (mg/kg)	Males mortality ^a	Time of death	Dose (mg/kg)	Females mortality ^a	Time of death
5000	0/5	-	4900 ^b	0/5	-

^a Number of animals which died/number of animals in dose group

^b Although the targeted dose rate for female rats was 5000 mg/kg, the actual average dose was 4900 mg/kg. This discrepancy is due, in part, to rounding errors associated with dose rate calculations and is not expected to change the mortality ratio.

Conclusion:

The oral LD₅₀ for Bensulfuron methyl 60WG was greater than 5000 mg/kg of body weight for both male and female rats. In accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification for acute oral toxicity is not required.

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

Toxicology and metabolism

B.6.11.2 Acute toxicity - percutaneous

46) *Acute dermal toxicity study with INF-5384-85 in rabbits . Sarver, J.W., 1986.*

Cross reference IIIA, 7.1.2./01

Guidelines:

US EPA 81-2

Deviations: None

GLP:

Yes

*The study is **acceptable***

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.s./kg

A single dose of Bensulfuron methyl 60WG moistened with distilled water to form a paste, was applied to the shaved, abraded skin of 5 male and 5 female New Zealand White rabbits at a dose of 2000 mg/kg. The application site was occluded for 24 hours after which the test substance was removed. The rabbits were observed for clinical signs, body weight effects, and mortality for up to 14 days following application. At the end of the 14-day observation period, rabbits were examined for gross pathological changes.

Findings:

No mortalities were observed (Table B.6. 7). Slight to moderate body weight losses (up to 7% of initial body weight) were observed 1 day after treatment. Erythema could not be evaluated in 2 of 5 male rabbits and in 4 of 5 female rabbits 1 day after treatment since the test material and gauze pads adhered to the skin. One of the 3 male rabbits had mild oedema 1-day post treatment. No erythema or oedema was observed in the other 3 rabbits that could be evaluated for skin irritation 1 day after treatment. One male rabbit exhibited mild erythema on days 2 and 3 post-treatment, and dry and cracked skin on day 3. No other clinical signs of toxicity or dermal irritation were observed through study termination. No test substance-related gross lesions were observed at necropsy.

dermal toxicity of Bensulfuron methyl 60WG

Dose (mg/kg)	Males mortality ^a	Time of death	Dose (mg/kg)	Females mortality ^a	Time of death
2000	0/5	-	2000	0/5	-

^a Number of animals which died/number of animals in dose group

Conclusion:

The dermal LD₅₀ for Bensulfuron methyl 60WG was greater than 2000 mg/kg body weight for both male and female rabbits. In accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification for acute dermal toxicity is not required.

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

Toxicology and metabolism

B.6.11.3 Acute toxicity - inhalation

An acute inhalation toxicity study was not required based on the stability or low attrition potential for the granule during shipping, the low acute inhalation toxicity of the active substance, and the low concentration of respirable particles in the formulation.

Bensulfuron methyl 60WG is a water-dispersible granular formulation (WG) consisting of 60% bensulfuron methyl, and 40% inert substances. This plant protection product is non-volatile. The vapour pressure of bensulfuron methyl is 2.8×10^{-12} Pa. This product will not be applied in a manner which generates a significant proportion of particles or droplets of diameter $<50 \mu\text{m}$ ($>1\%$ on a weight basis), nor will it be used in confined areas. The active substance demonstrated very low toxicity via the inhalation route [bensulfuron methyl $\text{LC}_{50} > 5.0 \text{ mg/L}$ (Burgess, B.A., 1983: Bensulfuron methyl EU Dossier, Annex IIA, Document M-II, Section 3, DuPont-6183 EU, Point 5.2.3). Since the formulated product and active substance also exhibit low toxicity by other exposure routes, Bensulfuron methyl 60WG is expected to have low acute inhalation toxicity. Thus, according to Directive 94/79/EC of Dec. 1994, an acute inhalation study was not required.

B.6.11.4 Acute toxicity - skin irritation

47) *Primary dermal irritation study with IN--F5384-85 in rabbits. Sarver, J.W., 1986.*

Cross reference IIIA, 7.1.4./01

Guidelines:

US EPA 81-5

Deviations: Treatment sites were abraded. However, there were also intact sites evaluated for irritation potential, which follows current guidelines

GLP:

Yes

*The study is **acceptable***

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.s./kg

Bensulfuron methyl 60WG was applied as a single 0.5 g dermal dose to the shaved intact and abraded skin of 6 male New Zealand White rabbits. There were two intact test sites and two abraded test sites on each rabbit. The test substance, moistened with approximately 0.9 mL of physiological saline, was applied to a 6 cm^2 area of skin. The application area was covered with a 1-inch gauze square, which was held in place with non-irritating tape and covered with rubber sheeting for a semi-occlusive dressing. The rabbits were exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated by the Draize method (1959) for signs of dermal irritation 24, 48 and 72 hours and up to 14 days after test substance removal.

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

Toxicology and metabolism

Findings:

No dermal irritation was produced in any of the intact sites (Table B.6.78). In some abraded sites, slight and moderate erythema was observed at 24 and 48 hours post treatment (Table B.6.79). Two rabbits had slight oedema at 24 hours post treatment. Mild erythema was observed in one abraded site at 72 hours but had subsided to slight erythema by day 6. Slight erythema persisted in this rabbit through study termination (day 14). The severity of the erythema observed in this abraded site may have been exacerbated by mechanical trauma from the abrader or from shaving. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.78: Individual and mean dermal irritation scores according to Draize (1959) – Intact sites

Animal no.	Erythema												Oedema											
	20352		20354		20355		20356		20357		20358		20352		20354		20355		20356		20357		20358	
24 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
48 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
72 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Day 6	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 7	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 8	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 9	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 10	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 13	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Day 14	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	
Mean scores 24-72 h	0.00												0.00											
Additional criteria specified in Directive 93/21/EEC Point 3.2.6.1 fulfilled: Yes/No																								
- Since these rabbits did not exhibit dermal irritation at 72 hours, they were removed from the study.																								

Table B.6.79: Individual and mean dermal irritation scores according to Draize (1959) – Abraded sites

Animal no.	Erythema												Oedema											
	20352		20354		20355		20356		20357		20358		20352		20354		20355		20356		20357		20358	
24 h	0	0	0	0	0	3	1	1	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
48 h	0	0	0	0	0	3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 6	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 7	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 8	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 9	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 10	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 13	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
Day 14	-	-	-	-	0	1	-	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-
mean scores 24-72 h	0.42												0.06											
Additional criteria specified in Directive 93/21/EEC Point 3.2.6.1 fulfilled: Yes/No																								

Conclusion:

Based on the mean degree of skin reaction observed at 24 to 72 hours, and according to the criteria specified in Directive 1995/45/EC, which refers to Directive 67/548/EEC for classification criteria, Bensulfuron methyl 60WG does not classify as a skin irritant.

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

Toxicology and metabolism

B.6.11.5 Acute toxicity - eye irritation

48) *Primary eye irritation study with IN-F5384-85 in rabbits. Henry, J.E., 1986.*

Cross reference IIIA, 7.1.5./01

Guidelines:

US EPA 81-4 and EEC 84/449 Annex V – Method B5 (1984)

Deviations: None

GLP:

Yes

The study is acceptable

Material and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.s./kg

49) *Supplement No. 1 to primary eye irritation study with Londax® 60 DF (IN-F5384-85) in rabbits. Henry, J.E., 1991*

Cross reference IIIA, 7.1.5./02

Guidelines:

US EPA 81-4 and EEC 84/449 Annex V – Method B5 (1984)

Deviations: None

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.s./kg

A single dose of 0.1 ml (equivalent to 77 mg) Bensulfuron methyl 60WG powder was administered into the lower conjunctiva sac of the right eye of 6 female New Zealand White rabbits. The eyes were not rinsed after introduction of the test substance. The conjunctiva, iris, and cornea of each treated eye were evaluated for evidence of irritation approximately 1, 24, 48, and 72 hours following administration of the test substance. Four rabbits were further examined at 7 and 14 days after administration of the test substance.

Findings:

The test substance produced generalised slight or mild corneal opacity, moderate iritis, mild conjunctival redness and slight or mild chemosis in all treated eyes. Five of 6 rabbits also had copious blood-tinged discharge. Biomicroscopic examinations revealed mild corneal opacity in 4 of 6 treated eyes and moderate or moderate to severe corneal opacity

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

Toxicology and metabolism

in the remaining two treated eyes. Although most ocular effects had resolved by day 7, corneal opacity persisted in 3 of 6 rabbits through day 7. However, in terms of eye irritation scores as applied by the classification criteria, the ocular lesions were less severe than those that would trigger classification. All treated eyes were normal by day-14 post application. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.80: Individual and mean eye irritation scores according to Draize (1959)

Cornea						
Animal no.	20437	20438	20451	20452	20455	20456
1 hour	1a	1a	1a	1a	1a	1a
24 hours	1	1	1	2	2	1
48 hours	1	1	1	2	2	1
72 hours	1	0	1	2	2	0
7 days	1	-	0	1	1	-
14 days	0	-	0	0	0	-
Overall Average of Means (24 - 72 h): 1.22						
Iris						
Animal no.	20437	20438	20451	20452	20455	20456
1 hour	1	1	1	1	1	1
24 hours	1	1	0	1	0	0
48 hours	0	0	0	1	0	0
72 hours	0	0	0	1	0	0
7 days	0	-	0	0	0	-
14 days	0	-	0	0	0	-
Overall Average of Means (24 - 72 h): 0.28						
Conjunctiva-redness						
Animal no.	20437	20438	20451	20452	20455	20456
1 hour	1	1	1	1	1	1
24 hours	1	1	1	1	1	1
48 hours	1	1	1	1	1	1
72 hours	1	0	1	1	1	0
7 days	0	-	0	0	0	-
14 days	0	-	0	0	0	-
Overall Average of Means (24 - 72 h): 0.89						
Conjunctiva-chemosis						
Animal no.	20437	20438	20451	20452	20455	20456
1 hour	1	1	1	2	2	2
24 hours	1	0	0	2	1	1
48 hours	1	0	0	1	0	0
72 hours	1	0	0	1	0	0
7 days	0	-	0	0	0	-
14 days	0	-	0	0	0	-
Overall Average of Means (24 - 72 h): 0.50						
Additional criteria specified in Directive 93/21/EEC Point 3.2.6.2 fulfilled: Yes/No						

^a Area = 4

Since these rabbits did not exhibit ocular irritation at 72 hours, they were removed from the study.

Conclusion:

Based on the mean degree of skin reaction observed at 24 to 72 hours, and according to the criteria specified in Directive 1995/45/EC, which refers to Directive 67/548/EEC for classification criteria. Bensulfuron methyl 60W does not classify as an eye irritant.

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

Toxicology and metabolism

B.6.11.6 Acute toxicity - skin sensitisation

50) *Bensulfuron methyl (DPX-F5384) 60WG: Dermal sensitization - Magnusson-Kligman maximization method. Moore, G.E., 2003.*

Cross reference IIIA, 7.1.6./02

Guidelines:

USEPA 870-2600 (1998)

Deviations: None

GLP:

Yes

*The study is **acceptable***

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-348

Purity: 600 g a.s./kg

The dermal sensitisation potential of Bensulfuron methyl 60WG evaluated by the Magnusson-Kligman Maximisation test in male Hartley albino guinea pigs. The study was conducted using three stages: preliminary irritation screens (intra dermal injection and topical application), a two-stage induction phase, and a challenge phase.

Preliminary irritation screens: For the intra dermal irritation screen, 4 guinea pigs each received 6 intra dermal injections (0.1 mL each) of 1, 3, or 5% of the test substance in distilled water, and 1, 3, or 5% of the test substance in an emulsion of Freund's Complete Adjuvant. For the topical irritation screen, 4 guinea pigs each received a topical application of 40% or 80% test substance diluted with distilled water, for 48 hours. The highest non-irritating concentration for topical exposure was determined in 4 guinea pigs by topically applying 25, 50, 75, or 80% of the test substance diluted in water for 24 hours.

Induction phase: The first induction phase involved 6 intra dermal injections (0.1 mL each) into the suprascapular area of each of 20 guinea pigs. These doses were comprised of pairs of injections of the test substance in distilled water, the test substance combined with Complete Freund's Adjuvant, and Adjuvant alone. A vehicle control group of 10 animals received injections of distilled water, distilled water combined with Complete Freund's Adjuvant, and Adjuvant alone. The second induction phase was conducted approximately one-week later. The test substance (60% w/w in distilled water, 0.5 g) or distilled water (0.5 g) was applied topically for 48 hours to the area encompassing the intra dermal injection sites. After patch removal, the test sites were cleansed of any residual test substance. Approximately one hour after the topical induction patches were removed, all animals were scored for erythema.

Challenge phase: Approximately 2 weeks later, 3 occluded topical applications of distilled water (0.5 mL), 80% test substance in distilled water (0.5 g), and 26.4% test substance (0.5 mL) in distilled water were applied to naïve sites for 24 hours. After removal of the patches, all test sites were cleansed of residual test substance. Approximately 24 and 48 hours after challenge patch removal, all animals were scored for a sensitisation response (erythema).

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

Toxicology and metabolism

Findings:

Results of irritation phase: A 5%w/w mixture of the test substance in distilled water was selected for intradermal induction. Due to severe irritation produced by the 80% w/w mixture and slight irritation produced by the 40% w/w mixture, 60% w/w of the test substance was selected for topical induction. The 80% w/w mixture was selected for the challenge phase.

Results of main study: The incidence and severity of the sensitisation response is presented in the tables below:

Table B.6.81: Incidence of skin reactions using the Maximisation Test

Substance tested	Test animals ^a 24 hours	Test animals ^a 48 hours	Control animals ^a 24 hours	Test animals ^a 48 hours
80% w/w test substance	0/20	0/20	0/10	0/10
24.6% w/w test substance	0/20	0/20	0/10	0/10
Distilled water	0/20	0/20	0/10	0/10

^a Animals with scores greater than 0.5

Table B.6.82: Severity of skin reactions using the Maximisation Test

Substance tested	Test animals 24 hours ^a	Test animals 48 hours ^a	Control animals 24 hours ^a	Test animals 48 hours ^a
80% w/w test substance	0.30	0.25	0.30	0.10
24.6% w/w test substance	0.10	0.08	0.10	0.05
Distilled water	0.0	0.0	0.0	0.0

^a Sum of the erythema scores divided by the number of animals evaluated

Conclusion:

Bensulfuron methyl 60WG does not possess skin sensitising potential under the conditions of the Maximisation test. Based on this negative response and in accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification for skin sensitisation is not required.

51) *Bensulfuron methyl (DPX-F5384) 60WG: Dermal sensitization test - Buehler Method. Moore, G.E., 2002. Cross reference IIIA, 7.1.6.01*

Guidelines:

OECD 406; OPTS 870.2600

Deviations: None

GLP:

Yes

The study is *acceptable*

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.s./kg

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

Toxicology and metabolism

Preliminary irritation testing was performed on four animals to determine appropriate concentrations of the test substance that could be used for both the induction and challenge phases. The test substance was diluted with distilled water to 25%, 50%, 75%, and 80% (0.4 g or mL) and applied topically for 6 hours. Approximately 24 and 48 hours after application, each site was evaluated for erythema.

For the main study, an 80% w/w mixture of the test substance in distilled water (0.4 g) was topically applied for 6 hours to 20 Hartley guinea pigs, once each week for a 3-week induction period. A vehicle control group (10 animals) was treated with distilled water during the induction phase. Twenty-seven days after the first induction dose, a challenge dose of the test substance (80% w/w in distilled water) and vehicle were applied to a naïve site on test guinea pigs. In addition, a test substance irritation group was included and treated with the test substance and vehicle. Approximately 24 and 48 hours after each induction and challenge dose, the test and irritation control animals were scored for erythema.

Table B.6.83: Incidence of skin reactions using the Buehler method

Substance tested	Test Animals 24 hours ^a	Test Animals 48 hours ^a	Control Animals 24 hours ^a	Test Animals 48 hours ^a
80% w/w test substance	0/20	0/20	0/10	0/10
Distilled water	0/20	0/20	0/10	0/10

^a Animals with scores greater than 0.5

Table B.6.84: Severity of skin reactions using the Buehler method

Substance tested	Test animals 24 hours ^a	Test animals 48 hours ^a	Control animals 24 hours ^a	Test animals 48 hours ^a
80% w/w test substance	0.03	0.00	0.00	0.00
Distilled water	0.00	0.00	0.00	0.00

^a Sum of the erythema scores divided by the number of animals evaluated

Conclusion:

Bensulfuron methyl 60WG does not possess skin sensitising potential under the conditions of the Buehler method. Based on this negative and in accordance with the provisions of Directive 1999/45/EC, which refers to Directive 67/548/EEC for classification criteria, classification for skin sensitisation is not required.

B.6.11.7 Supplementary studies

No supplementary studies are required to satisfy the data requirements for registration of Bensulfuron methyl 60WG.

B.6.11.7.1 Summary of acute toxicity

Table B.6.85: Summary of acute toxicity studies with Bensulfuron methyl 60WG

Parameter	Species	Result	Reference
Acute oral LD ₅₀	Rat	> 5000 mg/kg	Sarver 1986a
Acute dermal LD ₅₀	Rabbit	>2000 mg/kg	Sarver, 1986b
Acute skin irritation	Rabbit	Non irritant	Sarver, 1986c
Acute eye irritation	Rabbit	Non irritant	Henry, 1986
Skin Sensitisation: Buehler method	Guinea Pig	Non sensitising	Moore, 2002
Maximisation test	Guinea Pig	Non sensitising	Moore, 2003

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

Toxicology and metabolism

Bensulfuron methyl 60WG has no significant acute toxicity via the oral and dermal routes of exposure. Based on the low toxicity of the active substance, Bensulfuron methyl 60WG would not be expected to produce significant acute toxicity by the inhalation route of exposure. Bensulfuron methyl 60WG is not a skin or eye irritant and does not cause skin sensitisation.

B.6.12 Dermal absorption (Annex IIIA 7.3)

B.6.12.1 *In Vivo* Dermal absorption

52) *Dermal absorption of [¹⁴C]bensulfuron methyl in rats. Hardesty, P.T., 1990.*

Cross reference IIIA, 7.3./01

Guidelines:

USEPA 85-2

Deviations: None

GLP:

Yes

*The study is **acceptable***

Materials and methods:

Test Substance: ¹⁴C-DPX-F5384 technical, DPX-F5384 technical

Batch: [Phenyl (U)-¹⁴C] HOTC Number 320; F5384-B-1B K4788

Purity: 93%, >90% respectively

An *in vivo* skin absorption study was conducted to determine the dermal absorption, elimination and distribution of [¹⁴C]bensulfuron methyl equivalent residues into and through the skin of male CrI:CD[®]BR rats after application of Bensulfuron methyl 60WG formulation. Administered doses were nominally 0.08, 0.8 or 8 mg bensulfuron methyl per rat (0.0067, 0.067, or 0.67 mg per cm² skin, applied as 0.4, 4.0 and 40.0 mg/mL solutions, respectively), and the exposure durations were 1, 2, 4, 10, and 24 hours. The lowest concentration (0.4 mg bensulfuron methyl/mL) is slightly higher and considered representative of the typical spray-tank dilution of 0.3 mg/mL. Surrounding the dose skin site was a protective appliance that prevented ingestion and dispersion of the dermally-applied dose. Test animals were maintained individually in all-glass metabolism chambers, which allowed the separate collection of urine and faeces. Following the exposure period, rats were anaesthetized with methoxyflurane, the protective appliance removed, and the dose-site skin washed successively with soapy water and water. The dose-site was excised from the anaesthetised rats, blood withdrawn by cardiac puncture, and urine collected from the bladder. Carcass, excreta, blood, dose-site skin, the appliance, and skin washes were analysed for radioactivity.

Table B.6.86: Summary of dosing solutions

Dose	% Bensulfuron methyl	Specific activity $\mu\text{Ci}/\text{mg a.s.}$	Specific activity $\mu\text{Ci}/\text{mmol a.s.}$	% Radiochemical purity
0.08 mg/rat	52 $\pm$ 1	57.5 $\pm$ 0.0	23.6 $\pm$ 0.0	93 $\pm$ 1
0.8 mg/rat	45 $\pm$ 4	19.6 $\pm$ 1.1	8.04 $\pm$ 0.45	94 $\pm$ 2
8 mg/rat	60 $\pm$ 1	1.23 $\pm$ 0.02	0.50 $\pm$ 0.01	93 $\pm$ 2

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

Toxicology and metabolism

Findings:

Recovery of administered radioactivity averaged 93-96%, 99.5-99% and 99.3-98% for the 0.08, 0.8, and 8 mg per rat groups, respectively. The majority of the radioactivity, from 79% to 94% of the administered dose, was found in the dose-site skin washes (Table B.6.87). Radioactivity associated with the appliance averaged <10% for all exposure durations and concentrations. Radioactivity in the blood was very low. The highest value was 0.05% at the 1 hour time point for the 0.08 mg per rat dose. The range of carcass radioactivity levels were 0.36-0.72%, 0.28-1.14%, and 0.19-0.65% of administered radioactivity in the 0.08, 0.8, and 8 mg per rat groups.

Urine was the primary route of excretion in all groups with the exception of the 24-hour exposure duration for the 0.08 mg per rat group where urine and faecal elimination were similar. In all other groups, the faecal elimination was 0.1% of the applied doses, and the urinary elimination was <0.4%. For all dose levels, the amount of radioactivity excreted was greatest after 24 hours of exposure and there was a general upward trend in the amount excreted as the exposure duration increased.

The majority of the dose that was absorbed was found in the dose site skin (0.7% to 11.2%). The absorption did not increase with longer exposure. Dermal absorption of the applied dose, defined as the sum of radioactivity in the excreta, carcass, blood and dose-site skin at 10 and 24 hours after administration of the 0.08, 0.8, and 8 mg per rat dosages was 1.66%, 11.31%, and 4.68% for 10-hours and 7.18%, 7.96%, and 5.89% for 24 hours, respectively (Table B.6.88 and Table B.6.89). There was not a linear increase in absorption over time. The amount of administered dose that penetrated the skin (radioactivity in excreta, carcass and blood) for the 1-24 hour exposure period ranged from 0.77-1.47%, 0.59-1.40%, and 0.25-0.69% for the 0.08, 0.8, and 8 mg per rat dose groups.

Table B.6.87: Percent distribution of radioactivity in rats exposed to Bensulfuron methyl 60WG

Sample	Dose (mg/rat)	1 hour	2 hour	4 hour	10 hour	24 hour
Carcass	0.08	0.72	0.57	0.57	0.57	0.36
	0.8	0.54	1.14	0.42	1.05	0.28
	8	0.28	0.65	0.19	0.56	0.47
Blood	0.08	0.05	0.02	0.01	NA	NA
	0.8	0.01	0.01	0.01	NA	NA
	8	NA	NA	NA	NA	NA
Urine	0.08	0.18	0.17	0.21	0.38	0.79
	0.8	0.04	0.08	0.28	0.35	0.35
	8	0.04	0.04	0.06	0.09	0.12
Faeces	0.08	NA	0.01	NA	0.01	0.64
	0.8	NA	NA	NA	NA	0.09
	8	NA	NA	NA	NA	0.02
Dosed skin	0.08	3.54	2.10	0.97	0.70	5.39
	0.8	11.17	5.42	4.69	9.94	7.24
	8	3.87	3.79	3.21	4.03	5.28
Appliance	0.08	9.41	1.82	2.02	0.26	6.05
	0.8	1.51	3.25	3.32	5.33	1.03
	8	1.51	1.20	0.49	0.62	1.56
Skin wash	0.08	81.66	91.42	91.47	93.71	84.92
	0.8	86.66	87.98	86.98	78.50	89.09
	8	90.68	90.41	93.56	91.33	89.09

NA = not available

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Table B.6.88: Absorption of radiolabeled bensulfuron methyl (a.s.) by rats exposed to aqueous dilutions of Bensulfuron methyl 60WG equivalent to approximately 1.3- to 133-X field application solution concentration

Sacrifice time after application:	10h		24h	
	Percent of administered radioactivity (%)		Percent of administered radioactivity (%)	
	0.40 ^a mg/mL	40.0 ^b mg/mL	0.40 ^a mg/mL	40.0 ^b mg/mL
Absorption:				
Unabsorbed dose:				
skin washes	93.71	91.33	84.92	89.09
protective device ^a	0.26	0.62	6.05	1.56
Total unabsorbed dose:	93.97	91.95	90.97	90.65
Absorbable dose:				
application skin	0.70	4.03	5.39	5.28
Total absorbable dose:	0.70	4.03	5.39	5.28
Absorbed dose:				
Urine	0.38	0.09	0.79	0.12
Faeces	0.01	0	0.64	0.02
Whole blood	0	0	0	0
Other Tissues (not performed)	0	0	0	0
Carcass	0.57	0.56	0.36	0.47
Cage washes	0	0	0	0
Total absorbed dose:	0.96	0.65	1.79	0.61
Total recovered (material balance):	95.63	96.63	98.15	96.54

^a Equivalent to 0.08 mg a.s./rat

^b Equivalent to 8.0 mg a.s./rat

- Not reported

Conclusion:

The majority of the dose that was adsorbed was found in the skin dosage site (0.7%-11.2%). The percentage of dose actually penetrating the skin and available for absorption (i.e., trapped in skin) at 10-hours was less than 2%.

B.6.12.2 Summary and conclusions

The dermal absorption of bensulfuron methyl (a.s.) from the product (Bensulfuron methyl 60WG) was investigated under *in vivo* conditions in the rat. Tests were done with a 0.4 mg a.s./mL dilution, a dilution rate that slightly exceeds the maximum field use concentration (0.30 mg a.s./mL). This dilution will be utilised to estimate total dermal absorption from spray-tank dilutions (i.e., 1.7% at 10 hours; Table B.6.89). The highest concentration, 40.0 mg a.s./mL resulted in a total dermal absorption value of 4.7% at 10 hours. This value was used to estimate the mixer/loader exposures. Both values represent a combination of absorbed and absorbable bensulfuron methyl (i.e., the quantity that actually penetrates the skin and that trapped in the skin, and presumed available for subsequent absorption).

The results are summarised in Table B.6.89.

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Table B.6.89: Dermal absorption of bensulfuron methyl (a.s.) from Bensulfuron methyl 60WG and spray solution

		Bensulfuron methyl 60WG	Spray solution	
Study	Sample time	% Absorbed ^a	% Absorbed ^a	Reference
<i>In vivo</i> - rat	10 h	4.68	1.66	Hardesty, 1990
	24 h	5.89	7.18	

^a Includes quantity of bensulfuron methyl trapped in the skin and is therefore available for absorption.

B.6.13 Toxicological data on no active substances (Annex IIIA 7.4 and point 4 of the introduction)

This information is available on material safety data sheets (MSDS) found in Bensulfuron methyl EU Dossier, Document J, Part 3, DuPont-6174 EU.

B.6.14 Exposure data (Annex IIIA 7.2)

B.6.14.1.1 Operator exposure

B.6.14.1.1.1 Estimation of operator exposure

Bensulfuron methyl 60WG is an herbicide containing bensulfuron methyl as the active substance. Bensulfuron methyl 60WG is to be applied to rice. The maximum dose rate is 0.06 kg a.s./ha with a minimum application of volume of 200 L/ha.

For the purposes of risk assessment a systemic NOAEL of 19.9 mg/kg bw/day is chosen from the 1-year dog study. Using the NOAEL of 19.9 mg/kg bw/day and applying a default safety factor of 100 results in an AOEL of 0.2 mg/kg/day (absorbed dose). The metabolism study conducted in rats indicates that bensulfuron methyl is almost completely absorbed from the gastrointestinal tract, thus no correction for oral absorption is applied. *In vivo* dermal penetration data for Bensulfuron methyl 60WG indicate 4.7% for mixer/loader and 1.7% for applicator, respectively (see data point 7.3 in this document) for inhalation exposure 100% absorption value is utilised for operator risk assessment purposes.

Operator exposure to Bensulfuron methyl 60WG was estimated using the German and UK models, with the most conservative assumptions for dose rate and application volumes. Exposure estimates assuming the use of personal protective equipment (PPE), gloves for mixing, loading, and for application, were also carried out. Operator exposure (absorbed dose), as estimated by the models, was calculated as a proportion of the proposed AOEL of 0.2 mg/kg bw/day.

Estimation of operator exposure using the German Model

Operator exposure was estimated according to the German model as published in *Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection): Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992*. A summary of the estimated exposures is presented in Table B.6.90. Details of the estimated exposure calculations are presented in Appendix.2.

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Table B.6.90: German model operator exposure estimation for Bensulfuron methyl 60WG

Crop	Application method	Exposure (mg a.s./kg bw/day)		% of AOEL	
		No PPE	PPE ^a	No PPE	PPE ^a
Field crop 200 L/ha	Tractor mounted equipment 0.06 kg a.s./ha	0.0024	0.0007	1.2	0.33

Note: German model = geometric mean values

^a Gloves for mixing/ loading and gloves for application

Estimation of operator exposure using the UK POEM model:

Operator exposure was estimated according to the revised Predictive Operator Exposure Model (UK POEM) 2003. The model assumes a workload of 50 ha per day for field crops, e.g. cereals. For rice culture in Europe, a considerably lower workload is appropriate. For the present modelling a conservative workload of 20 ha/day has been utilised. A summary of the estimated exposures calculated for bensulfuron methyl is presented in Table B.6.91. Details of the estimation of exposure calculations are presented in Appendix 2.

Table B.6.91: UK POEM operator exposure estimation for Bensulfuron methyl 60WG

Crop	Application method	Exposure (mg a.s./kg bw/day)		% of AOEL	
		No PPE	PPE ^a	No PPE	PPE ^a
Field crop 200 L/ha	Tractor mounted/trailed boom sprayer, hydraulic nozzles, 0.06 kg a.s./ha	0.0140	0.0057	7	2.85

^a Gloves for mixing/loading and gloves for application

B.6.14.1.1.2 Summary of operator exposure estimations

Operator exposure and risk estimations for field crop applications have been made using the German and UK models. A fully acceptable margin of safety for operators was demonstrated using both models with or without the use of PPE (gloves). Considering the conservative assumptions of the models, the overall estimated risk to operators is considered to be fully acceptable when loading, mixing, and during application by tractor mounted equipment in rice, according to good agricultural practices and label specified directions for use.

B.6.14.1.2 Measurement of operator exposure

The risk assessment detailed above indicates that it is unlikely that exposures to Bensulfuron methyl 60WG through agricultural use will exceed the proposed AOEL. Therefore, no measurements of field operator exposures were conducted.

B.6.14.2 Bystander exposure

Bensulfuron methyl 60WG has a relatively low use rate, and is applied by ground-directed tractor-mounted hydraulic nozzles that produce large non-respirable spray droplets. Bensulfuron methyl and Bensulfuron methyl 60WG have low volatility and when considered along with the low inhalation toxicity, supports negligible inhalation exposures and risks to bystanders. Potential dermal exposure is estimated using drift estimates based on data published by

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EPPO (Ganzelmeier, et. al., 1995). Assuming a bystander may be within 10 meters of the spray application, the data shows that for field crops (e.g., rice), the drift estimate at this distance is 0.3%.

The following bystander exposure assumptions were made: The maximum application rate for Bensulfuron methyl 60WG of 100g/ha in rice was used and the spray was evenly distributed over the treated area (10,000 m²), equating to an application rate of 10 mg/m². A bystander located 10 meters from the field might receive 0.3% drift, thus the deposition could reach 0.03 mg/m²; if one-half the body surface (approximately 1 m²) is uncovered, the skin deposition would be 0.018 mg bensulfuron methyl; Using the 1.7% value from the *in vivo* dermal penetration study as human skin absorption value, the absorbed dose would be 0.00032 mg bensulfuron methyl; For a 60 kg body weight, the exposure would be 0.0000051 mg/kg bw/day of bensulfuron methyl.

A comparison with the proposed AOEL of 0.2 mg/kg bw/day for Bensulfuron methyl shows there is a margin of safety >40,000. This gives assurance that it is unlikely that any adverse health effect may result in bystanders exposed to bensulfuron methyl from agricultural use of Bensulfuron methyl 60WG. Accordingly, measurement of bystander exposure is not required.

B.6.14.3 Worker exposure

B.6.14.3.1 Estimation of worker exposure

Bensulfuron methyl 60WG herbicide is labelled for use on field crop, rice. Plant metabolism studies carried out in rice demonstrated that bensulfuron methyl was rapidly metabolised indicating that residues would not be found in grain at harvest. Bensulfuron methyl residues were below quantifiable or detectable levels of the analytical methods used to measure residues in rice grain at harvest (<LOQ of 0.020 or <LOD of 0.007 mg/kg). In addition rice is mechanically harvested, which further reduces the potential for exposure. Therefore, it is unlikely that any significant exposure to Bensulfuron methyl 60WG will occur. For these reasons, the establishment of a re-entry interval for workers is not necessary.

B.6.14.3.2 Measurement of worker exposure

A study to determine the actual exposure of workers to bensulfuron methyl has not been conducted. Estimations of worker exposure (point 7.2.3.1) indicated adequate margins of safety (toxicity/exposure ratios) to ensure the safety of workers harvesting rice.

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.

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B.6.15 Reference 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 5.1) IIA, 5.1./01	Cornelissen, K.S., Lewis, C., Hopkins, R.	1990	(¹⁴ C)-Bensulfuron methyl: Absorption, distribution, metabolism and excretion in the rat [REDACTED] AMR 964-87 GLP: Yes Published: No	N	DuPont
(IIA 5.1) IIA, 5.1./02	Cornelissen, K.S., Lewis, C., Hopkins, R.	1992	(¹⁴ C)-Bensulfuron methyl: Absorption, distribution, metabolism and excretion in the rat [REDACTED] AMR 964-87, Amendment No. 1 GLP: Yes Published: No	N	DuPont
(IIA 5.1) IIA, 5.1./03	Cornelissen, K.S., Lewis, C., Hopkins, R.	1993	(¹⁴ C)-Bensulfuron methyl: absorption, distribution, metabolism and excretion in the rat [REDACTED] AMR 964-87, Amendment No. 2 GLP: Yes Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.1./01	Redgate, D.	1984	Median lethal dose (LD ₅₀) of IN-F-5384- 52 in rats - EPA proposed guidelines [REDACTED] HLR 239-84 GLP: Not specified Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.2./01	Gargus, J.L.	1983	Acute skin adsorption LD ₅₀ test on rabbits with technical DPX-F5384 [REDACTED] HLO 225-83 GLP: Not applicable Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.3./01	Burgess, B.A.	1983	Inhalation median lethal concentration (LC ₅₀) on IN-F5384 by EPA protocol [REDACTED] HLR 332-83 GLP: Yes Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.3./02	Kinney, L.A.	1987	Inhalation median lethal concentration (LC ₅₀) of INF-5384-38 by EPA protocol [REDACTED] HLR 332-83, Addendum GLP: Yes Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.4./01	Gargus, J.L., Cline, E.J.	1984	Skin irritation test on rabbits for EPA pesticide registration [REDACTED] HLO 187-84, Revision No. 1 GLP: No, Published: No	N	DuPont

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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 5.2) IIA, 5.2.5./01	Gargus, J.L., Cline, E.J.	1984	Eye irritation test in rabbits - EPA pesticide registration ████████████████████ HLO 174-84 GLP: No Published: No	N	DuPont
(IIA 5.2) IIA, 5.2.6./01	Armondi, S.	1991	Closed-patch repeated insult dermal sensitization study (maximization method) with DPX-F5384-122 in guinea pigs ████████████████████ HLO 628-91 GLP: Yes Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./01	Bolte, H.F.	1986	A one-year feeding study in dogs with H14793 ████████████████████ HLO 35-86, Addendum No. 1 GLP: No Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./02	Daly, I.W.	1986	One-year feeding study in dogs with DPX-F5384 (5 Volumes) ████████████████████ HLO 35-86 GLP: No Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./03	Hirano, M.	1984	DPX-F5384: 13-week oral subacute toxicity study in mice ████████████████████ 5384 TOX 1 GLP: No Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./04	O'Neal, F.O.	1986	Ninety-day feeding and one-generation reproduction study in rats with benzoic acid, 2-((((4,6-dimethoxy-2- pyrimidinyl) amino)-carbonyl) amino) sulfonylmethyl)-methyl ester, (INF-5384) ████████████████████ HLR 186-83 GLP: No Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./05	Stadler, J.C.	1986	Three-month feeding study in dogs with INF-5384 ████████████████████ HLR 3-85, Revision No. 1 GLP: No Published: No	N	DuPont

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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 5.3) IIA, 5.3.2./06	Stula, E.F.	1988	One-year feeding study in dogs with DPX-F5384 (5 Volumes) [REDACTED] HLO 35-86, Supplement No. 1 GLP: No Published: No	N	DuPont
(IIA 5.3) IIA, 5.3.2./07	Stula, E.F.	1986	Supplementary tables required for registration in Japan [REDACTED] HLR 186-83, Supplement No. 1 GLP: No Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.1./01	Arce, G.T.	1987	Mutagenicity evaluation of INF-5384-7 in <i>Salmonella typhimurium</i> DuPont Haskell Laboratory HLR 609-81, Revision No. 2 GLP: Yes Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.1./02	Covell, D.L.	1988	<i>In-vitro</i> evaluation of IN-F5384-122 for chromosome aberrations in human lymphocytes [REDACTED] HLR 26-88 GLP: Yes Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.1./03	Chromey, N.C.	1984	CHO/HGPRT assay for gene mutation DuPont Haskell Laboratory HLR 212-84 GLP: No Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.1./04	Chromey, N.C., McCooley, K.T.	1984	Unscheduled DNA synthesis/rat hepatocytes <i>in vitro</i> [REDACTED] HLR 278-84 GLP: No Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.1./05	Vlachos, D.A.	1986	<i>In vitro</i> evaluation of INF-5384-52 for sister chromatid exchange in chinese hamster ovary (CHO) cells DuPont Haskell Laboratory HLR 683-86 GLP: Yes Published: No	N	DuPont
(IIA 5.4) IIA, 5.4.2./01	Farrow, M.G.	1984	<i>In vivo</i> bone marrow chromosome study in rats [REDACTED] HLO 210-84 GLP: Yes Published: No	N	DuPont

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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, 5.4) IIA, 5.4.2./02	Vlachos, D.A.	1987	<i>In vivo</i> bone marrow chromosome study in rats treated with INF-5384-52 (H# 14,793-02) [REDACTED] HLO 210-84, Addendum No. 1 GLP: Yes Published: No	N	DuPont
(IIA, 5.5) IIA, 5.5./01	Maita, K.	1985	DPX-F5384: 24-month oral chronic toxicity and oncogenicity study in mice (10 Volumes) [REDACTED] (IET) (Japan) TX 5384-1-86 GLP: Yes Published: No	N	DuPont
(IIA, 5.5) IIA, 5.5./02	Slone, T.W.	1988a	Combined chronic toxicity/oncogenicity study with IN-F5384 long-term feeding and two-generation, four-litter reproduction study in rats supplemental appendix (Two Volumes) [REDACTED] HLR 662-85, Supplement No. 1 GLP: Yes Published: No	N	DuPont
(IIA, 5.5) IIA, 5.5./03	Slone, T.W.	1988b	Combined chronic toxicity/oncogenicity study with IN-F5384 long-term feeding and two-generation, four-litter reproduction study in rats supplemental tables and appendix [REDACTED] HLR 662-85, Supplement No. 3 GLP: Yes Published: No	N	DuPont
(IIA, 5.5) IIA, 5.5./04	Stadler, J.C.	1986	Long-term feeding and two-generation, four-litter reproduction study in rats with INF-5384 (four volumes) [REDACTED] HLR 662-85 GLP: No Published: No	N	DuPont
(IIA, 5.5) IIA, 5.5./05	Stula, E.F.	1988	Combined chronic toxicity/oncogenicity study with IN-F5384 long-term feeding and two-generation, four-litter reproduction study in rats supplemental tables and appendix [REDACTED] HLR 662-85, Supplement No. 2 GLP: Yes Published: No	N	DuPont

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(IIA 5.6) IIA, 5.6.1./01	Mullin, L.S.	1989	Reproductive and fertility effects with INF5384-122 multigeneration reproduction study in rats (Two Volumes) [REDACTED] HLR 430-89 GLP: Yes Published: No	N	DuPont
(IIA 5.6) IIA, 5.6.2./01	Lamontia, C.L.	1999	Developmental toxicity study of INF-5384-38 by gavage in the rat [REDACTED] HLR 1-84, Revision No. 2 GLP: No Published: No	N	DuPont
IIA, 5.6.2./02 (IIA 5.6)	Zellers, J.E.	1985	INF-5384: Developmental toxicity study in rabbits dosed by gavage on days 7-19 of gestation [REDACTED] HLR 704-85 GLP: Yes Published: No	N	DuPont
(IIA 5.6) IIA, 5.6.2./03	Zellers, J.E.	1987	Teratogenicity study of INF-5384 in rabbits dosed by gavage on days 7-19 of gestation [REDACTED] HLR 704-85, Addendum No. 1 GLP: Yes Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./01	Bentley, K.S.	1994	Mutagenicity testing of IN-J290-17 in the <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> plate incorporation assay DuPont Haskell Laboratory HLR 319-94, Revision No. 2 GLP: Yes Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./02	Finlay, C.	1994	Acute oral toxicity study with IN-J290- 17 in male and female rats [REDACTED] HLR 348-94 GLP: Yes Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./03	Fisher, G.A., Sarver, J.W.	1985b	Approximate lethal dose (ALD) of IN-B6895-2 in rats [REDACTED] HLR 577-85 GLP: No Published: No	N	DuPont

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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 5.8) IIA, 5.8.1./04	Rickard, L.B.	1985a	Mutagenicity evaluation of INB-6895-2 in <i>Salmonella typhimurium</i> (Ames assay) DuPont Haskell Laboratory HLR 613-85 GLP: Yes Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./05	Rickard, L.B.	1985b	Mutagenicity evaluation of INR-9419-1 in <i>Salmonella typhimurium</i> DuPont Haskell Laboratory HLR 653-85 GLP: Yes Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./06	Sarver, J.W.	1985	Approximate lethal dose (ALD) of IN-R9419-1 in rats [REDACTED] HLR 525-85 GLP: No Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./07	Summers, J.C.	1983	Mutagenicity evaluation in <i>Salmonella</i> <i>typhimurium</i> DuPont Haskell Laboratory HLR 292-83, Revision No. 1 GLP: No Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./08	Wylie, C.N., Burgess, B.A.	1983	Approximate lethal dose (ALD) of IN-N5297-1 in rats [REDACTED] HLR 315-83 GLP: No Published: No	N	DuPont
(IIA 5.8) IIA, 5.8.1./09	Wylie, C.N.	1983	Ten-dose oral subacute test in rats (IN-J290-1) [REDACTED] HLR-260-83 GLP: No Published: No	N	DuPont
(IIA 5.9) IIA, 5.9.1./01	O'Neal, F.O.	1996	Medical examination summaries of employees manufacturing and formulating DuPont sulfonyleurea herbicides: Manati, Puerto Rico facility DuPont Barley Mill Plaza CHLORI-METS/GEN 1 GLP: Not applicable position paper Published: No	N	DuPont
(IIIA 7.1) IIIA, 7.1.1./01	Sarver, J.W.	1986	Acute oral toxicity study with IN-F5384- 85 in Rats [REDACTED] HLR 680-86 GLP: Yes Published: No	N	DuPont

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

Toxicology and metabolism

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
(III A 7.1) III A, 7.1.2./01	Sarver, J.W.	1986	Acute dermal toxicity study with INF-5384-85 in rabbits [REDACTED] HLR 708-86 GLP: Yes Published: No	N	DuPont
(III A 7.1) III A, 7.1.4./01	Sarver, J.W.	1986	Primary dermal irritation study with IN- F5384-85 in rabbits [REDACTED] HLR 712-86 GLP: Yes Published: No	N	DuPont
(III A 7.1) III A, 7.1.5./01	Henry, J.E.	1986	Primary eye irritation study with IN-F5384-85 in rabbits [REDACTED] HLR 618-86 GLP: Yes Published: No	N	DuPont
(III A 7.1) III A, 7.1.5./02	Henry, J.E.	1991	Supplement No. 1 to primary eye irritation study with Londax® 60 DF (IN- F5384-85) in rabbits [REDACTED] HLR 618-86, Supplement No. 1 GLP: Yes Published: No	N	DuPont
(III A 7.1) III A, 7.1.6./01	Moore, G.E.	2002	Bensulfuron methyl (DPX-F5384) 60WG: Dermal sensitization test - Buehler Method [REDACTED] DuPont-9898 GLP: Yes Published: No	Y	DuPont
(III A 7.1) III A, 7.1.6./02	Moore, G.E.	2003	Bensulfuron methyl (DPX-F5384) 60WG: Dermal sensitization - Magnusson-Kligman maximization method [REDACTED] DuPont-12391 GLP: Yes Published: No	Y	DuPont
(III A, 7.3) III A, 7.3./01	Hardesty, P.T.	1990	Dermal absorption of [¹⁴ C]bensulfuron methyl in rats [REDACTED] AMR 1527-89 GLP: Yes Published: No	N	DuPont
	Frost, L. O'Neal, F.	2006	Position Paper Evaluation of toxicological relevance of the bensulfuron methyl metabolite IN- J0290 Published: No	N	DuPont