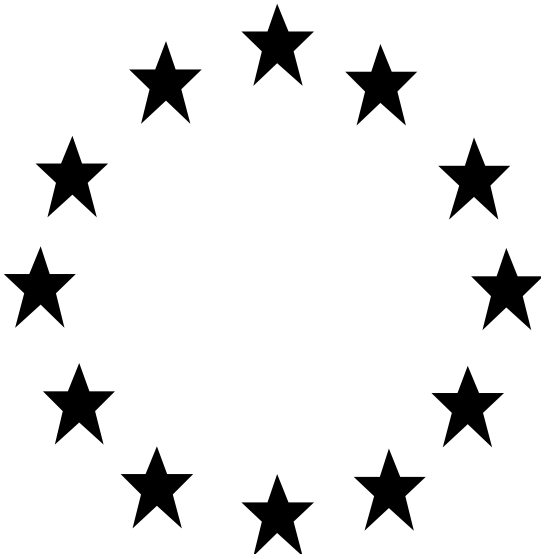

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State The Netherlands for the new active substance

SPIRODICLOFEN

as referred to in Article 8(1) of Council Directive 91/414/EEC

June 2009

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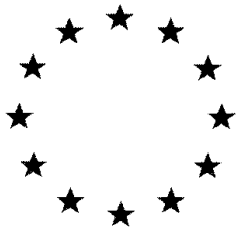
European Commission



SPIRODICLOFEN

ADDENDUM B6 B7

EPCO-MEETINGS 28-29

Rapporteur Member State: The Netherlands

June 2005, revised September 2006

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

CONTENTS

Volume 3

B.6 TOXICOLOGY AND METABOLISM

Introduction

This toxicological dossier contains studies with the test substance spirodiclofen, which is known under the code name BAJ 2740. Spirodiclofen is the substance under notification, intended to be placed on Annex I. In the Evaluation Meeting (Febr. 2005) several issues were raised, mainly concerning the lack of detail in the summaries for an independent evaluation by the MS. In some cases the setting of the NOAEL was questioned. In total 1 data requirement and 14 open points were identified in the Evaluation Table dated 09.03.2005. The issues are addressed either in the Reporting Table or in the June 2005 addendum.

In this September 2006 revised addendum several additional issues are discussed (and marked in green):

- In the Expert Meeting (June/July 2005) several of these issues were discussed, and the RMS was to present the dermal absorption values as agreed upon, and the revised operator, worker and bystander exposure scenarios in an addendum.
- Moreover, the notifier submitted a developmental neurotoxicity study in June 2005, which is also presented in this revised addendum. This study has not been peer-reviewed.
- Finally, in the evaluation table a new point was raised by EPCO 30 experts regarding the new specification from the full-scale production. This point is evaluated in this revised addendum in B.6.8 further toxicological studies.

B.6.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS)

B.6.1.1 Toxicokinetic studies

In the Evaluation Meeting (febr. 2005) it was indicated that some information on the evaluated tissues in study 1 was not cleary described in the DAR (open point 2.3 in Evaluation Table). Therefore, the study summary was copied from the DAR and additional information was added and highlighted.

STUDY 1

Characteristics

Reference	:	I. Andersch, J. Köster, (2000a)	exposure	:	single low and high dose and repeated low dose
type of study	:	Absorption, distribution, excretion and metabolism	doses	:	1, 2, 100 mg/kg bw
year of execution	:	1997-2000	vehicle	:	0.5% carboxymethyl cellulose
test substance	:	[Dihydrofuranone-3-^{14}C] BAJ 2740 (spirodiclofen) (purity >89%)	GLP statement	:	yes
Route	:	Oral (by gavage)	guideline	:	In accordance with OECD 417
Species	:	rat (Wistar Hsd/Cpb: Wu)	acceptability	:	Acceptable
group size	:	4-6/male/dose/time point 1 experiment 4 female/dose/time point			

Study design

Rats received a single oral dose (1, 2 or 100 mg/kg bw) of ^{14}C-BAJ 2740 (spirodiclofen) or a repeated oral dose (14 daily doses of 2 mg/kg bw unlabelled spirodiclofen followed by a single oral dose of 2 mg/kg bw ^{14}C-spirodiclofen on day 15). Urine was collected at 4, 8, 24 h and daily after exposure to the labeled test compound. In the bile-duct cannulated rats urine and bile were collected at 1, 2, 3, 4, 6, 8 and 24 h after dosing. Feces was collected once per day. Following administration of ^{14}C-spirodiclofen, blood was sampled from the tail vein, at 0.08, 0.16, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8 24, 32, 48 h and daily thereafter. Excretion of radiolabel in air was assessed at 4, 8, 24 and 48 h after administration in one experimental group. At sacrifice organs and tissues were dissected and, after combustion, radioactivity levels were measured by LSC (LOQ of 0.0023 μg/g). From urine-, feces- and bile samples metabolites were isolated by solid phase extraction and HPLC, followed by identification by LC-MS, LC-MS/MS and NMR. An overview of the experiments is presented in table B.6.1.1.

Table B.6.1.1 Overview of experiments on kinetics and metabolism of spirodiclofen (BAJ 2740).

Experiment	Dose (mg/kg bw)	Animals	No. of doses	sampling[1]	Time of Sacrifice (hr) after last dose
ADME	100	4 male	1	P, U, F	168
	2	4 male	1	U, F, A	48
	2	4 male	1	P, U, F	48 [2]
	2	4 female	1	P, U, F	48 [2]
	2	4 male	15	P, U, F	48 [2]
	1	6 male	1	U, F, B	24

1 radioactivity measured in P=plasma, U=urine, F=feces, A=expired air, B=bile

2 At sacrifice the following organs and tissues samples were collected and prepared for measurement of radioactivity levels: erythrocytes, plasma, spleen, gastrointestinal tract, liver, kidney, renal fat, adrenal gland, skeletal muscle, bone (femur), heart, lung, brain, thyroid gland, skin, carcass. Additionally in males radioactivity levels were measured in testes and in females in uterus and ovary.

Results

In male and female rats plasma radioactivity levels rose quickly following single oral administration of a dose of 2 mg/kg bw, reaching maximum concentrations of 2.1-2.7 µg eq/g after 2-6 h in males, and after 1.5-4 h in females. In the 100 mg/kg bw group a peak plasma level of 51.3 µg eq/g was reached after 8 h, i.e. slightly later than after the low dose administration. In the low dose groups calculated half-lives for elimination of radioactivity from plasma were 4.2 h (males) and 3.4 h (females), in the males of the 100 mg/kg bw group calculated half-live was 3.1 h.

Recovery of radioactivity, as percentage of dose administered ranged from 89-107. Excretion of administered radioactivity was fast. Following administration of 2 mg ^{14}C-spirodiclofen/kg bw to male rats more than 99 % of recovered radioactivity was excreted within 48 h, i.e. 64-66 % in urine (59-61 % after 24h) and 33-35 % in feces (31-32% after 24 h). As compared to males, in females relatively more radioactivity was excreted in the urine (76 % in 48 h) and less in feces (24% in 48 h). After administration of 100 mg ^{14}C-spirodiclofen/kg bw to male rats 100 % of the recovered radioactivity was excreted within 168 h, i.e. 36.7% via urine and 63.3 % via feces. The relatively high amount of excretion in the feces indicates incomplete absorption at this high dose. Following administration of 1 mg ^{14}C-spirodiclofen/kg bw to bile-duct cannulated male rats 62.8 % of recovered radioactivity was

excreted within 24 h, i.e. 22.8% in urine, 28.7 % in feces and 11.3 % in bile. The presence of a substantial amount of radioactivity in feces of bile-duct cannulated rats suggests that also at this low dose absorption of spirodiclofen is incomplete. In view of the radioactivity levels in urine and bile it can be assumed that in male rats more than 64 % of spirodiclofen, administered at a dose of 2 mg/kg bw, is absorbed, although it has to be noted that in the bile experiment the urinary excretion was low. In female rats the absorption may be even higher. After administration of 2 mg ^{14}C-spirodiclofen/kg bw less then 0.05 % of radioactivity was expired in air within 48 h.

Levels of radioactivity in tissues and organs were low. In male rats treated with a single dose of 2 mg ^{14}C-spirodiclofen/kg bw, at 48 h after administration the highest equivalent concentrations were observed in liver (0.049 µg/g), kidneys (0.024 µg/g), plasma (0.015 µg/g), gastro-intestinal tract (0.0135 µg/g) and skin (0.0107 µg/g). Concentrations in erytrocytes, spleen, skeletal muscle, heart, lung and carcass were below 0.01 µg eq/g. Testis concentrations were 0.0029 µg/g, and concentrations in renal fat, adrenal gland, bone femur, brain and thyroid gland were below LOD. In male rats treated for 15 days with spirodiclofen at a daily dose of 2 mg/kg bw, the relative distribution of radioactivity over the tissue was similar to that of male rats after single treatment. However, the concentrations in organs and tissue were approximately 4 times lower (liver 0.0105 µg/g, kidneys 0.0064 µg/g, plasma 0.0037 µg/g, gastro-intestinal tract 0.0061 µg/g and carcass 0.0020 µg/g). Concentrations in erytrocytes, heart and lung were below 0.0020 µg eq/g. Testis concentrations were 0.0008 µg/g, and no radioactivity (< LOD) was found in spleen, renal fat, adrenal gland, skeletal muscle, bone femur, brain, thyroid gland and skin. In females rats treated with a single dose of 2 mg ^{14}C-spirodiclofen/kg bw, at 48 h post administration concentrations were even 5 to 15 times lower than in males after a single dose, although the relative distribution of radiolabel was similar. In these females only in liver (0.0047 µg/g), kidney (0.0044 µg/g), gastrointestinal tract (0.0033 µg/g), plasma (0.0010 µg/g) and lung (0.0009 µg/g) radiolabel concentrations were above level of detection. The concentration in erytrocytes was below LOD. No radioactivity (< LOD) was found in spleen, renal fat, adrenal gland, uterus, ovary, skeletal muscle, bone femur, brain, thyroid gland, skin, and carcass.

The study authors report that no measurable radioactivity was detected at 168h post administration of 100 mg ^{14}C-spirodiclofen/kg bw (data not shown).

Metabolites were isolated by solid phase extraction and HPLC, followed by identification by LC-MS, LC-MS/MS and NMR. BAJ 2740 (spirodiclofen) M01, M02, M03, M06 and M16 were included as reference compounds. In total 14 metabolites of spirodiclofen were identified (see B.6.1.2) and 2 metabolites were partially identified. Between 78-90 % of the administered radioactivity could be identified. A proposed metabolic pathway is depicted in B.6.1.3. The quantitative excretion of the main metabolites in feces, urine and bile, expressed as percentage of recovered radioactivity is presented in table B.6.1.2. In male rats treated with 100 mg ^{14}C-spirodiclofen/kg bw mainly the parent compound and BAJ-enol (M01) were found in the feces. In feces of rats treated with 2 mg ^{14}C-spirodiclofen/kg bw less than 5 % was unmetabolized spirodiclofen. In these rats the main metabolites in feces and urine were BAJ-enol (M01) and the equatorial (e) and axial (a) 3- and 4-hydroxy-BAJ-enol isomers (M02 and M03). There was a striking sex difference with respect to the excretion of BAJ-enol in urine: in males treated once with 2 mg^{14}C-spirodiclofen/kg bw 2.3-3.8 % of the recovered radioactivity corresponded to BAJ-enol while in females receiving the same treatment this was 54.8 %.

Accordingly, in urine the levels of the 3- and 4-hydroxy-BAJ-enol isomers were high (55.1-57.4%) in males and lower (17.3%) in females. There were no obvious differences in quantitative distribution of metabolites between male rats receiving one dose of spirodiclofen or male rats repeatedly dosed with spirodiclofen. In bile of male rats the main metabolites were OH-enol-glucuronide and 3- and 4-hydroxy-BAJ-enols. No parent compound was detected in bile. In feces of bile-duct cannulated male rats 28.7 % of recovered radioactivity was found, of which only 0.6 % corresponded with the parent compound. The main metabolites were 3- and 4-hydroxy-BAJ-enols and BAJ-enol. Furthermore, the MA-cyclohexyl ester and dichlorobenzoic acid were almost exclusively found in feces. This suggests that substantial metabolisation of spirodiclofen (BAJ 2740) may take place inside the gastrointestinal tract.

Table B.6.1.2 Quantitative excretion of BAJ 2740 (spirodiclofen) and metabolites, expressed as % recovered radioactivity.

Dose	100 mg/kg bw		2 mg/kg bw		2 mg/kg bw		2 mg/kg bw		1 mg/kg bw		
sex	male		male		female		male #		male		
duration of experiment	168h		48h		48 h		48 h		24 h		
	feces	urine	feces	urine	feces	urine	feces	urine	feces	urine	bile
BAJ2740 (spirodiclofen)	16.6	n.d.	2.0-4.8	n.d.	0.7	n.d.	2.5	n.d.	0.6	n.d.	n.d.
BAJ-enol	16.9	5.9	5.1-7.5	2.3-3.8	4.9	54.8	4.0	5.2	13.9	0.0	0.2
4-hydroxy-BAJ-enol (e)	1.6	8.1	3.1-5.3	14.2-16.6	7.6	8.9	6.2	16.2	6.6	12.8	0.9
4-hydroxy-BAJ-enol (a)	n.d.	3.5	0.6-1.0	6.5-7.5	0.6	2.7	0.6	6.1	0.2	3.2	0.5
3-hydroxy-BAJ-enol (e)	1.8	13.2	2.7-3.4	29.2-30.3	1.1	4.5	3.2	32.1	0.7	4.5	3.8
3-hydroxy-BAJ-enol (a)	0.4	1.8	0.5-0.7	4.1-4.2	0.7	1.3	0.6	5.2	0.3	0.9	0.4
OH-BAJ-enol-glucuronide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.2
dichlorobenzoic acid	1.6	0.1	0.5-1.3	n.d.	0.5	n.d.	0.7	n.d.	0.1	n.d.	n.d.
MA-cyclohexyl ester	7.8	n.d.	2.6-3.3	n.d.	1.4	n.d.	1.7	n.d.	2.0	n.d.	n.d.

#14 daily doses of 2 mg/kg bw unlabelled spirodiclofen followed by a single oral dose of 2 mg/kg bw ^{14}C-Spirodiclofen on day 15.

Acceptability

The purity of the unlabeled compound is not reported. It is stated in appendix 17 to the report that the data from rat no. 873 in the bile-duct experiment were excluded from calculation “due to lower activity in the bile”. The appendix, however, shows that these data fall well within the range of the data from the other rats. According to the present reviewers this exclusion will have no major consequences for the interpretation of the data.

The study is considered acceptable for the overall toxicological evaluation.

B.6.3 SHORT-TERM TOXICITY

In the Evaluation Table (open point 2.4) the MS are to confirm the relevant NOAEL for short-term studies at an expert meeting. Moreover, UK requested more transparency in the 28d study summaries (reporting table 2(7)), commented on the subchronic studies, and the notifier submitted some position

papers. Furthermore the notifier was requested to fulfil a data requirement (2.1) regarding historical data in a subchronic feeding study in rats.

B.6.3.1 28-day oral studies

STUDY 1 28-day oral study in rats

The notifier submitted a position paper, proposing a higher NOAEL than that proposed by the RMS. However, in the DAR the study was considered not acceptable by the RMS, and therefore the study was not considered an essential study.

STUDY 2 28-day oral study in dogs

Questions were raised in the Evaluation Meeting on missing details in the study summary. Therefore the study summary is copied from the DAR, and more detailed information is added and highlighted.

Characteristics

reference/notifier	:	Wetzig, H., A. Romeike, E. Sander (2001) (revised report to report PH 29421 from 03-01-2000)	exposure	:	28 days
type of study	:	4 week oral toxicity study (range finding study)	doses	:	0, 400, 2000 and 10000 ppm (equal to 11.3, 65.5 and 284.5 mg/kg bw/day)
year of execution	:	1996	vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 99.1%)	GLP statement	:	no
Route	:	Oral (diet)	guideline	:	Not in accordance with OECD 409
Species	:	Dog, Beagle	acceptability	:	Acceptable as range-finding study
group size	:	2/sex/dose	**NOAEL**	:	11.3 mg/kg bw/day (range-finding study)

Study design

Deviations from OECD guideline 409: 2/sex/dose (instead of 4/sex/dose), age of the dogs 24-46 weeks (instead of maximally 9 months = 39 weeks), body weight range 6.9 to 15.4 kg.

Results

The results of the study are summarized in table 6.3.1.2. Details on organ weight are specified in Table 6.3.1.2.a

Table 6.3.1.2

Dose (ppm)	0	400	2000	10000	dr
	m/f	m/f	m/f	m/f	
Mortality	none				
Clinical signs[1]	No toxicologically relevant effects				
Body weight	No toxicologically relevant effects				
Food consumption[2]	No toxicologically relevant effects				
Water consumption	No toxicologically relevant effects				

Dose (ppm)	0	400	2000	10000	dr
	m/f	m/f	m/f	m/f	
Haematology					
-ery			d	d	
-Hb			d	d	
-Ht			d	d	
-lymphocytes (%)				d	
-thromboplastin time				i	
Clinical chemistry					
-ASAT				i	
-ALAT			i	i	
-AP				i	
-GLDH				i	
-LDH				i	
-cholesterol				d	
-triglycerides				d	
-protein				d	
-albumin				d	
-Fe				d	
-T_4				d	
-albumin (electrophoresis)				d	
-α_1-globulin				i	
-β-globulin				i	
-γ-globulin				i	
Urinalysis					
-volume				d	
Liver tissue					
N-Dem (mU/g)	105	135	214 i	259 i	
O-Dem (mU/g)	20.6	24.4	32.8 i	34.0 i	
P_{450} (nmol/g)	21.4	23.7	32.4 i	40.8 i	
-ECOD (nmol/g*min)	20.3 / 21.9	38.8 i / 28.6	86.8 i / 48.4 i	89.7 i / 54.4 i	dr
-ALD (nmol/g*min)	21.3 / 19.2	22.2 / 28.6	25.4 i / 26.3 i	38.2 i / 29.9 i	dr
-EH (nmol/g*min)	2691 / 2188	2641 / 1938	4167 i / 2477	3692 i / 2508	
-Glu-T^3 (nmol/g*min)	2072 / 2755	2374 / 2222	2802 i / 2909	3366 i / 2687	dr
Organ weights					
-liver			$i^{a/r}$	$i^{a/r}$	
-kidneys			$i^{a/r}$	$i^{a/r}$	
-ovaries				$i^{a/r}$	
-uterus			$i^{a/r}$	$i^{a/r}$	
-adrenals			$i^{a/r}$	$i^{a/r}$	dr
-brain			i^{r}	i^{r}	dr
Pathology					
macroscopy	No toxicologically relevant effects				
microscopy					
liver					
-periportal single cell necrosis				4	
testes					
-Leydig cell vacuolation			2	2	
-Leydig cell hypertrophy/activation				1	
-immature testes/prostate				1	
Epididymides					
-massive oligo-/aspermia, slight spermic debris				1	
Adrenal glands					
-cytoplasmic vacuolation cortex	1	1	4	4	
jejenum					
Vacuolation superficial					

Dose (ppm)	0	400	2000	10000	dr
	m/f	m/f	m/f	m/f	
mucosal epithelial cells			1	3	

dr dose related
i/d increased /decreased
a/r absolute /relative organ weight
[1] in the results section, the study authors mention one animal to be judged as slim in the high dose group. However, in the table of the revised report, all animals were judged to be normal, whereas in the original report this animal was judged to be meager.
[2] one animal of the highest dose group showed reduced feed intakes throughout the study period and week –1, according to the study authors probably due to the low bw (6.9 kg).
[3] Increase observed in males only.

Table 6.3.1.2a Organ weight of several organs

Dose (ppm)	0	400		2000		10000	
	m+f	m+f		m+f		m+f	
Terminal body weight (kg)	11.63	12.88		11.78		11.00	
Liver							
- absolute (g)	390.50	464.25	19%	467.50	20%	429.25	10%
- relative (g/kg BW)	33.5	36.12	8%	39.97	19%	39.79	19%
Kidneys							
- absolute (g)	55.75	68.75	23%	63.50	14%	65.50	17%
- relative (g/kg BW)	4.78	5.35	12%	5.50	15%	6.10	28%
Ovaries							
- absolute (g)	1.000	1.100	10%	0.900	-10%	1.300	30%
- relative (g/kg BW)	0.0700	0.0886	27%	0.0844	21%	0.1259	80%
Uterus							
- absolute (g)	4.5	5.0	11%	6.0	33%	5.0	11%
- relative (g/kg BW)	0.3	0.403	34%	0.525	75%	0.467	56%
Adrenals							
- absolute (g)	1.248	1.488	19%	1.615	29%	1.560	25%
- relative (g/kg BW)	0.108	0.116	7%	0.141	31%	0.158	46%
Brain							
- absolute (g)	75.25	78.75	5%	84.00	12%	78.50	4%
- relative (g/kg BW)	6.598	6.125	-7%	7.397	12%	7.679	16%

Acceptability

The study was considered acceptable as range finding study.

Conclusions

Treatment-related effects were seen in de mid- and high dose groups. Since only 2m/2f were exposed in each dose group, results from males and females were considered together, and results are only indicative for the effects observed. Treatment-related effects were observed on haematological parameters. Changes in clinical biochemical determinations indicate treatment-related effects on liver and immune system. Effect on the liver was confirmed by induction of liver enzymes and increased liver weight in the mid- and high dose groups, and periportal single cell necrosis in the high dose

group, indicating the liver as target organ. In addition to the liver, other target organs were sex organs (increased organ weights, histopathological examination), adrenals (increased organ weights, histopathological examination) and jejenum (histopathological examination).
Based on the effects observed in the mid- and high-dose groups, the NOAEL in this (range finding) study is, in accordance with the opinion of the study authors, set at 400 ppm, equal to 11.3 mg/kg bw/day.

B.6.3.2 28-day dermal studies

STUDY 1 28-day dermal study in rats

The notifier submitted a position paper, proposing a higher NOAEL than that proposed by the RMS. However, the RMS does not agree and the reaction of the RMS on the notifier's comments were already incorporated in the reporting table dated 04.03.2005, see point 2(29). To possibly aid the discussion, the study summary is copied from the DAR, and more detailed information is added and highlighted.

Characteristics

reference/notifier	:	Krötinger, F., E. Sander (1999)	exposure	:	22 applications/28 days, 6 h/day, semi-occlusive
type of study	:	4-week dermal toxicity study, limit test	doses	:	0 and 1000 mg/kg bw/day
year of execution	:	1998	vehicle	:	The substance was applied as solid onto wet gauze pad
test substance	:	BAJ 2740 (spirodiclofen, purity 97.9%, fine crystalline powder)	GLP statement	:	yes
Route	:	dermal	guideline	:	In accordance with OECD 410
Species	:	Rat, Wistar HsdCpb:WU	acceptability	:	Acceptable as screeening test
group size	:	5/sex/dose	**NOAEL**	:	<1000 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 410, with the following deviaton: the substance was applied as solid onto wet gauze pad. Since it is stated that 1000 mg/kg is applied to 30.25 cm^2, the applied dose is less than 10% of the body surface, based on a default body surface of 400 cm^2.

Doses are based on a range-finding study with female rats (10 applications/14 days).

Results

The results of the study are summarized in table 6.3.2.1

Table 6.3.2.1

Dose (mg/kg bw/day)	0		1000		dr
	m	f	m	f	
Mortality	None				
Clinical signs	No toxicologically relevant effects				
Body weight				i[1]	
Food consumption				d[2]	
Local skin findings	No toxicologically relevant effects				
Haematology					
-Hb (g/l)	162	147	154 ds	146	
-Ht (l/l)	0.544	0.488	0.511 ds	0.478	
Clinical chemistry					
-ALAT			ds		
-Triglycerides (mmol/l)	1.36±0.29	0.61±0.20	0.85±0.34 ds	0.62±0.17	
Organ weights					
-adrenals[3]			d[ar]		
absolute (mg)	43	67	38 (-12%)	65	
relative (mg/100g bw)	15	32	14 (-7%)	30	
Pathology					
Macroscopy	No toxicologically relevant effects				
Microscopy	No toxicologically relevant effects				

dr dose related
i/ d increased/ decreased
ds statistically significantly decreased compared to the controls
r relative organ weight
[1] the body weights were significantly higher at day 7 and day 21
[2] food consumption was slightly decreased during weeks 1-3 in females, when expressed as mg/kg bw/d; there was no difference with control when expressed as mg/animal/d. The observed statistically significant decreased food consumption in males observed at day 7 is considered incidental.
[3] adrenal weights (absolute and relative) were higher (factor 2) in females compared to males

Acceptability

The study was considered acceptable as screening test, since effects were observed at the limit dose.

Conclusions

At the dose level tested, females showed a increased body weight compared to controls, whereas food consumption was lower in the dosed females. It should be noted that several females in the control group hardly gained weight during the study period.

In males, Ht and Hb were significantly decreased. The study author considered these effects incidental, since control values of these parameters are high, and values of the dose group are in the biological range of this species. However, the RMS considered the effects relevant. In addition to these effects, ALAT was significantly decreased in exposed males. Also in males, a significantly decrease in triglycerides was observed. The study author considered these effects incidental, since values of the dose group are in the biological range of this animal. However, substance-related effects on triglycerides were observed in several studies, and are considered toxicologically relevant. The

observed difference of a factor 2 between male and female control values for triglycerides were not explained by the study author, nor was the observed significant difference in adrenals weights between males and females discussed by the study author. Adrenal weights of males in the 1000 mg/kg bw group were decreased by ~~10~~12% and this is considered substance related.
Since effects were observed on haematological and clinical biochemical parameters and adrenal weights in the exposed group, the NOAEL systemic for dermal exposure is <1000 mg/kg bw.
There were no local effects in rats after 28 days dermal application of spirodiclofen at the dose level tested.

B.6.3.3 Semi-chronic oral studies

STUDY 1 13-week feeding study in mice

The notifier submitted a position paper, proposing a higher NOAEL than that proposed by the RMS. A MS also commented on the proposed NOAEL (see point 2(8) in the reporting table). However, the RMS does not agree and the reactions of the RMS were already incorporated in the reporting table dated 04.03.2005, see 2(30). To possibly aid the discussion, the study summary is copied from the DAR, and the grading of the liver hypertrophy is added and highlighted.

Characteristics

reference/notifier	:	Leser, K.H., A. Romeike (1998)	exposure	:	13 weeks
type of study	:	13 week oral toxicity study	doses[1]	:	0, 100, 1000 and 10000 ppm (equal to 15.3, 163.8 and 1629.9 mg/kg bw/day in males and 30.1, 233.6 and 2685.2 mg/kg bw/day in females)
year of execution	:	1996	vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 99.1%)	GLP statement	:	yes
route	:	Oral (diet, with 1% peanut oil)	guideline	:	In accordance with OECD 408
species	:	mouse, CD-1	acceptability	:	acceptable
group size	:	10/sex/dose	**NOAEL**	:	<15.3 mg/kg bw/day

[1] Dose levels were based on on the results of a pilot study with CD-1 mice in which 5/sex/dose animals were dosed with 0, 100, 1000 and 10000 ppm during 4 weeks (study no. T4060765, 1996).

Study design

The study was performed in accordance with OECD guideline 408 (deviation: no ophthalmoscopy performed). Remark: Females are exposed to higher doses, expressed in mg/kg bw/day, compared to males.

Results

The results of the study are summarized in table 6.3.3.1.

Table 6.3.3.1

Dose (ppm)	0		100		1000		10000		dr
	m	f	m	f	m	f	m	f	
Mortality	none								
Clinical signs	No toxicologically relevant effects								
Body weight					d		d	d	
Food and water consumption	No toxicologically relevant effects								
Ophthalmoscopy	Not performed								
Haematology									
-Hb								ds	
-Ht								d	
Urinalysis	Not performed								
Clinical chemistry[1]									
- cholesterol							d	ds	
Organ weights[2, 3]									
-adrenals						i[a/r]		i[a/r]	f
-spleen[4]								i[r]	
-kidneys					ds[a,r]		ds[a,r]		
-testes					i[a/r]		i[a/r]		m
-ovaries								i[r]	
Pathology									
macroscopy	No toxicologically relevant effects								
microscopy									
liver									
-centrilobular hepatocellular hypertrophy			3/10		3/10		6/10	2/10	
grade 2			3/3		3/3		5/6	2/2	
grade 3							1/6		
-periportal cytoplasmic vacuolisation							1/10	3/10	
Adrenal glands									
-cytoplasmic vacuolation						6/10	8/9	10/10	
-degeneration of cortical cells								9/10	
-mononuclear infiltrate								9/10	
Testes[5]									
-hypertrophy/activation Leydig cells	1/10		1/10		9/10		10/10		
-hypertrophy/activation Leydig cells, severity							i		
-vacuolation Leydig cells							7/10		

dr dose related
d/i decreased/ increased
ds decreased significantly/ increased significantly
a/r absolute/ relative organ weight

[1] plasma glucose determination was performed in non-fasting animals.

[2] notable is the observed higher relative weights of brain, adrenals and spleen in female animals compared to males.

[3] the observed significantly decreased liver weight (a, r) of male mice dosed 1000 ppm is considered incidental, since no effect on liver weight was observed in the highest dose group.

[4] mean spleen weight in male controls were high and showed a great SD, due to one animal with a high spleen weight.

[5] male sex organs (testes, epididymides, prostates, seminal vesicles) were re-examined, based on the observed Leydig cell alterations in a subacute and a subchronic study in dogs. Results of the reexamination were presented in a first amendment in the study.

Acceptability

The study was considered acceptable.

Conclusions

Adrenal weights were (not significantly, dose related) increased in females. Histopathologic examination of the adrenal glands showed increased cytoplasmic vacuolation in the 1000 ppm (f) and 10000 ppm (m/f) groups.

Absolute and relative weights of the testes of mice of the two highest dose groups were dose-related (not statistically significant) increased. Histological examination of the testes showed hypertrophy/activation of Leydig cells at an increased incidence in male mice dosed 1000 and 10000 ppm spirodiclofen. In the highest dose group, this finding was accompanied by an increased severity and vacuolation of Leydig cells.

Histological examination of the liver showed increased centrilobular hepatocellular hypertrophy in males of all dose groups and in females of the highest dose group, in addition to increased periportal cytoplasmic vacuolisation in the highest dose group (m/f).

The observed effects on liver, adrenal glands and testis are likely substance related. Based on the observed increased centrilobular hepatocellular hypertrophy in males of all dose groups, the NOAEL is < 100 ppm. The LOAEL for semi-chronic toxicity in mice is, in accordance with the opinion of the study author, set at 100 ppm, equal to 15.3 mg/kg bw/day.

STUDY 2 14-week feeding study in rats

The notifier submitted historical control data (Hartmann, 2005) for a better evaluation of the adrenal cortical vacuolation observed in males at all doses (see data requirement 2.1 in Evaluation Table).

The summary of the study is copied from the DAR and details on the adrenal cortical vacuolation are added and highlighted.

Characteristics

reference/notifier	:	Wirnitzer, U., A. Romeike (1998)	exposure	:	14 weeks (4 weeks for immunotox)
type of study	:	14 week oral toxicity study with 4 week recovery phase	Doses[1]	:	0, 100, 500, 2500 and 12500 ppm (0, 6.6, 32.1, 166.9, 851.4 mg/kg bw/day for males and 0, 8.1, 47.1, 215.3 , 995.8 mg/kg bw/day for females)
year of execution	:	1996	vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 99.1%)	GLP statement	:	yes
route	:	Oral (diet, with 1% peanut oil)	guideline	:	in accordance with OECD 408
species	:	Rat, Wistar (Hsd Cpb:WU)	acceptability	:	acceptable
group size	:	10/sex/dose (5/sex/dose for immunotox)	**NOAEL**	:	~~< 6.6~~ 8.1 mg/kg bw/day

[1] doses are based on results of a subacute study with female rats (0 to 5000 ppm).

Study design

The study was performed in accordance with OECD guideline 408, with the following deviations: sensory reactivity to stimuli of different types was not investigated; glucose was determined in blood of non-fasting animals.

For immunotoxicological investigations satellite groups of only 5/sex/dose were orally exposed to spirodiclofen only for 4 weeks. Results of this 4-weeks exposure study are presented as an additional toxicity study in section B.6.8. No immunototoxicological investigation was performed after 13 weeks of exposure.

Results

The results of the study are summarized in table 6.3.3.2. Details on incidence and grading of small cortical vacuolation in the adrenal gland are presented in Table 6.3.3.2a.

Table 6.3.3.2

Dose (ppm)	0		100		500		2500		12500		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	none										
Clinical signs	No toxicologically relevant effects										
Body weight (gain)[1]							d	d	ds	ds	m, f
Food consumption[2]									ds	ds	
Water consumption									d	d	
Ophthalmoscopy	No toxicologically relevant effects										
Urinalysis											
-volume									is		
-density									ds		
Haematology											
-leukocytes[3]							d		d	ds	m
-ery's										is	
-Hb									d		
-MCV										ds	
-MCH										ds	
-thrombocytes									ds		
-Hepato Quick (anticoagulation)							is	is	is	is	m, f
-lymphocytes (%)									d	d	
-segm (%)									i	i	
-eos (%)									i	is	
Clinical chemistry											
-AP							is		is	is	m
-GLDH[4]									ds	d	
-Cholesterol							ds		ds	ds	m
-Triglycerides								d	ds	ds	f
-Bili-t							ds		ds	ds	m
-Protein									ds	d	
-Phosphate									ds		
-Chloride										ds	
-TSH								is		is	f
Organ weights											
-liver									ds[a]	ds[a]	
-spleen							d[a]/ ds[r]		d[a/r]	ds[a/r]	

Dose (ppm)	0		100		500		2500		12500		dr
	m	f	m	f	m	f	m	f	m	f	
-adrenals									i[a]/ is[r]	is[a]/[r]	
-testes									i[r]		
-thymus									d[r]		
Pathology											
macroscopy	No toxicologically relevant effects										
microscopy											
Liver											
-reduced glycogen content	-	-	-	-	-	-	-	-	-	4/10	
Adrenals											
-small cortical vacuolation	5/10	4/10	9/10	3/10	8/10	8/10	10/10	8/10	10/10	10/10	
-grading	0.7	0.6	1.5	0.5	1.1	1.4	3.3	1.6	3.3	3.8	
-mixed cortical vacuolation	7/10	-	7/10	-	6/10	-	9/10	-	10/10	-	
-grading	1.6		1.2		0.9		1.2		2.9		
Duodenum											
-epithelial vacuolation	-	-	-	-	-	-	-	-	2/10	1/10	
Jejenum											
-epithelial vacuolation	-	-	-	-	-	-	8/10	7/10	8/10	7/10	
Ileum											
-epithelial vacuolation	-	-	-	-	-	-	1/10	-	-	2/10	

dr dose related
ds/is statistically significantly decreased/increased.
d/i decreased/increased.
a/r absolute/relative organ weight
- no abnormality detected
1 body weights of satellite groups were more affected.
2 food intake was significantly reduced during the first 4 days of treatment at 2500 and 12500 ppm in both sexes. During further treatment daily food intake was occasionally significantly reduced at 12500 ppm at both sexes. During the recovery period food intake in females was increased.
3 Observed at weeks 13, effect also observed (not per se statistically different) at week 5.
4 control values between male/females differed, without explanation of the study author. In addition, a great variance was observed in the female values. However, without explanation of the study author, the observed decrease in females in the highest dose group is considered relevant.

Table 6.3.3.2a Incidence and grading of small cortical vacuolation in the adrenal gland

Males

	0 ppm	100 ppm	500 ppm	2500 ppm	12500 ppm
No. examined	10	10	10	10	10
Small cortical vacuolation					
Grade 1	3	4	5		1
Grade 2	2	4	3	1	1
Grade 3		1		5	3
Grade 4				4	4
Grade 5					1
Total no. of tissues affected	**5**	**9**	**8**	**10**	**10**
Average grade/no. of animals per group	**0.7**	**1.5**	**1.1**	**3.3**	**3.3**
Average grade/no. of tissues affected	**1.4**	**1.7**	**1.4**	**3.3**	**3.3**

Females

	0 ppm	100 ppm	500 ppm	2500 ppm	12500 ppm
No. examined	10	10	10	10	10
Small cortical vacuolation					
Grade 1	2	1	3	2	
Grade 2	2	2	4	4	2
Grade 3			1	2	1
Grade 4					4
Grade 5					3
Total no. of tissues affected	**4**	**3**	**8**	**8**	**10**
Average grade/no. of animals per group	**0.6**	**0.5**	**1.4**	**1.6**	**3.8**
Average grade/no. of tissues affected	**1.5**	**1.7**	**1.8**	**2.0**	**3.8**

The submitted historical data for small cortical vacuolation in the male rat indicated that in 9 studies, including three with a recovery period, the average grade/no. of animals per group was between 0.1 and 1.1 (in 4 cases, the average grade was >0.7) and the average grade/no. of tissues affected was between 1.0 and 2.0 (in 6 cases, the average was >1.4) (Hartmann, 2005).

Acceptability

The study was considered acceptable.

Conclusions

Since immunotoxicologal investigation was performed after 4 weeks of exposure instead of after 14 weeks, the results of those investigations are not considered in this 14 week oral toxicity study, but evaluated as a separate study.

Test substance induced changes were mainly observed at and above 2500 ppm, and included decreased bw and leukocyte number, increased coagulation time, AP and TSH and decreased cholesterol, triglycerides and bilirubin concentrations. Absolute and relative weight of spleen was decreased. Histopathological examination showed increased incidence of epithelial vacuolation in jejenum (m, f) and increased incidence and severity of adrenal cortical vacuolation in males dosed 100 ppm and above, whereas in females this effect was observed at and above 500 ppm. Additional submitted historical data for male rats indicated that the observed cortical vacuolation in males at 100 and 500 ppm was within the range of historical data, and was therefore not considered to be an adverse effect. No historical data for females were presented. During the recovery period, effects were observed on body weight, ASAT, AP, cholesterol, triglycerides, protein, albumin, Cl, T3, T4 and TSH.

Based on the observed increased incidence and severity of adrenal cortical vacuolation in females ~~of all dose groups~~ at 500 ppm and above, the ~~L~~NOAEL in this study is 100 ppm, equal to ~~6.6~~ 8.1 mg/kg bw/day.

~~Remark: The RMS does not subscribe the opinion of the study author that males tolerated the test substance without effects up to and including 500 ppm and females 100 ppm.~~

STUDY 4

1-year oral toxicity study in dogs

Unfortunately, this study is summarised in the DAR in section B.6.5.1 Chronic toxicity and carcinogenicity. However, a 1-year study in dogs is considered semi-chronic, and should have been included in B.6.3.

Questions were raised in the Evaluation Meeting on missing details in the study summary. Therefore the study summary is copied from the DAR, and detailed information on the organ weights and T3 concentrations is added and highlighted.

Characteristics

reference/notifier	:	Wetzig, H., Chr. Rühl-Fehlert (2001)	exposure	:	52 weeks
type of study	:	1-year toxicity study	doses	:	0, 20, 50, 150, 500/600[1] ppm (0, 0.57, 1.45, 4.54. 16.9 mg/kg bw/day)
year of execution	:	1998/1999	vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 97.8%)	GLP statement	:	yes
route	:	Oral (diet)	guideline	:	According to OECD 452
species	:	Beagle dog	acceptability	:	acceptable
group size	:	4/sex/dose	**NOAEL**	:	1.45 mg/kg bw/day

1 The concentration in the 500 ppm group was increased to 600 ppm after about 3 weeks of exposure.

Study design

The study was in accordance with OECD guideline 453. In line with the small number of animals per group only mean values and standard deviations were calculated. For the parameters N-DEM, O-DEM, P450 and triglycerides a statistical evaluation was done for males and females/group together.

Results

The results of the study are summarized in table 6.5.1.3. Details of T3 concentrations in females and weights of heart, adrenals, liver and testes are specified in Table 6.5.1.3a and 6.5.1.3b

Table 6.5.1.3

Dose (ppm)	**0**		**20**		**50**		**150**		**600**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none										
Clinical signs	No toxicologically relevant effects										
Body weight (gain)	No toxicologically relevant effects										
Food consumption	No toxicologically relevant effects										
Ophthalmoscopy	No toxicologically relevant effects										
Haematology[a]	No toxicologically relevant effects										
Urinalysis	No toxicologically relevant effects										
Clinical chemistry[b]											
- ALAT									d		
- AP					i		i		i	d	m
- GLDH							d		d	d	

Dose (ppm)	0		20		50		150		600		dr
	m	f	m	f	m	f	m	f	m	f	
- Glucose			i		i		i		i		
- Cholesterol[c]			i	d	i	d	i		i	d	
- Potasium									d		
- Ferrum									d		
- T3				i		i		i		i	
- T4								i		i	f
- TBC									i		
Organ weights[d]											
- heart				i[a,r]		i[a,r]	i[a,r]	i[a,r]	i[a,r]	i[a,r]	
- lung							i[a,r]		i[a,r]		m
- adrenals			i[a,r]		i[a,r]		i[a,r]	i[a,r]	i[a,r]	i[a,r]	f
- brain									d[r]	d[r]	
- kidneys						i[a,r]		i[a,r]	i[a]	i[a,r]	
- liver			i[a,r]		i[a,r]		i[a,r]		i[a,r]		
- thyroid							i[a,r]		i[a,r]		
- testes			i[a,r]		i[a,r]		i[a,r]		i[a,r]		
- prostate									i[a,r]		
- epididymides					i[a,r]		i[a,r]		i[a,r]		
- pancreas					i[a]	i[a,r]	i[a]	i[a,r]	i[a]	i[a,r]	
- thymus										i[a,r]	
- uterus/oviduct										d[a,r]	
Liver examinations											
- N-DEM					i		i	i	i	i	m, f
- O-DEM					i		i		i	i	m
- P450									i	i	
Pathology											
Macroscopy	No toxicologically relevant effects										
Microscopy											
Duodenum											
- desquamation, epith.									2/4		
Liver											
- cytopl. inclusion					1/4		1/4		2/4		
- pigment								1/4		2/4	
Testes											
- vacuolat. Leydig cells									4/4		
- hypertrop. Leydig cells									1/4		
- focal tubular degener.							1/4		1/4		
Adrenals											
- vacuolation Z. fasc.	1/4	1/4	2/4	1/4			4/4	3/4	4/4	4/4	
- vacuolation Z. glom.								1/4		1/4	

dr dose related
a/r absolute/relative organ weight
[a] Hb and Ht in males of all dose groups lower; however: SD of the control group was large, and the observed decrease was not dose related. Fe in blood of males of the dosed groups were also slighly, not dose related, decreased.
[b] in females dosed 150 ppm, deviating values (compared to the other dose groups) were observed for GLDH, CK, glucose, cholesterol and triglycerides
[c] control cholesterol values differed for males and females (3 vs 6), whereas such difference could not be observed in historical values. The study author gives no explanation.
[d] ovaries and uterus/oviduct of females dosed 50 ppm were about half the weight of the ovaries of the other groups, with no explanation of the study author.

Table 6.5.1.3a T3 concentrations in females in nmol/l

Dose (ppm)	0	20	50	150	600
wk –3	2.07	1.76	1.81	1.97	1.79
wk 3	1.51	1.56	1.67	1.48	1.47
wk 6	1.61	1.77	1.66	1.75	1.78
wk 12	1.69	1.84	1.56	1.73	1.55
wk 26	1.42	1.68	1.58	1.75	1.52

wk 39	1.19	1.54	1.37	1.28	1.19
wk 52	1.49	2.27	1.85	1.88	1.82
Mean wk 3-52	1.49	1.78	1.62	1.65	1.56

Table 6.5.1.3b Weights of heart, adrenals, liver and testes in grams

Dose (ppm)	0	20		50		150		600	
Heart (females only)									
- absolute	106.0	114.0	8%	126.5	19%	118.3	12%	119.0	12%
- relative	7.48	8.45	13%	9.17	23%	8.24	10%	8.10	8%
Adrenals (males only)									
- absolute	1.638	1.763	8%	1.835	12%	1.738	6%	1.973	20%
- relative	0.1064	0.1208	14%	0.1191	12%	0.1154	8%	0.1232	16%
Liver (males only)									
- absolute	438.0	510.0	16%	548.8	25%	525.5	20%	552.5	26%
- relative	28.95	34.85	20%	35.83	24%	34.79	20%	34.24	18%
Testes (males only)									
- absolute	20.43	23.95	17%	22.30	9%	26.55	30%	29.13	43%
- relative	1.380	1.636	19%	1.438	4%	1.767	28%	1.790	30%

Acceptability

The study was considered acceptable.

Conclusions

In the two lower dose groups, several clinical biochemical parameters and relative and absolute organ weights were affected, mainly without dose-relationship (i.e. effects are more or less constant or decreasing over the exposure groups). In the absence of associated histopathological changes in these dose groups and the fact that histopathological changes are only observed for a few organs at much higher doses, the observed effects at the lower doses are considered not clearly adverse. The adrenals are regarded to be the critical target organ. At and above 150 ppm, increased adrenal weights were observed accompanied by increased vacuolation of the adrenals, an effect which was also observed in several other toxicological studies with different species. Based on the effects observed on the adrenals in the two highest dose groups, the NOAEL in this study is, in accordance with the opinion of the study author, 50 ppm, equal to 1.45 mg/kg bw/day.

B.6.3.4 Summary

In the summary on short-term toxicity studies copied from the DAR, the NOAEL in the 14-week oral study in rats is adapted and the 1-year oral study in dogs is added.

Table 6.3.4.1 Subacute toxicity studies

Test substance	Duration, route	Species	NOAEL (mg/kg bw/day	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Notifier
Spirodiclofen (BAJ 2740)	28 days, oral (range finding)	dog	11.3	65.5	Effects on haematology, increased ALAT, increased enzyme activities in liver tissue (N-DEM, O-DEM, P450, ECOD, ALD, EH, Glu-T), decreased kidney weight, increased weights of uterus, adrenals and brains, Leydig cell vacuolation, cytoplasmic vacuolation adrenal cortex.	H. Wetzig, A. Romeike, E. Sander (2001)
Spirodiclofen (BAJ 2740)	28 days, dermal	rat	< 1000	1000	Decreased Hb, Ht, ALAT and triglycerides and decreased relative absolute adrenal weight	F. Krötlinger, E. Sander (1999)

In a 28 day oral toxicity study with dogs, treatment-related effects were observed on haematological parameters. Also in dogs, changes in clinical biochemical parameters indicate treatment-related effects on liver and the immune system. Effect on the liver was confirmed by induction of liver enzymes in the mid-and high-dose groups and liver periportal cell necrosis in the high-dose group. In addition to the liver, effects were also observed on adrenals and jejenum (organ weight and cortical vacuolation) and sex organs (changed organ weights and vacuolation of Leydig cells of the testes). The NOAEL in this range finding study is 11.3 mg/kg bw/day.

A dermal exposure study during 28 days with rats showed effects on haematological parameters, ALAT, triglycerides and adrenal weight in the exposed group. Similar effects were also observed in oral toxicity studies and considered substance-related. A NOAEL in this study could not be established, and the LOAEL systemic for dermal exposure is 1000 mg/kg bw/day. There were no local effects observed at the dose level tested.

Table 6.3.4.2 Semichronic toxicity studies

Test substance	Duration, route	Species	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Notifier
Spirodiclofen (BAJ 2740)	13 weeks, oral	mouse	<15.3	15.3	Centrilobular hepatocellular hypertrophy in males	K.H. Leser, A. Romeike (1998)
Spirodiclofen (BAJ 2740)	14 weeks, oral	rat	8.1	47.1	Adrenal cortical vacuolation accompanied by increased grading of vacuolation	U. Wirnitzer, A. Romeike (1998)
Spirodiclofen (BAJ 2740)	8 weeks, oral	dog	<2.9	2.9	Increased AP, increased organ weights of liver, thyroid, adrenals, thymus and pancreas, decreaed prostate weight, increased cytoplasmic vacuolation of the adrenal cortex	H. Wetzig, E. Hartmann (2001)
Spirodiclofen (BAJ 2740)	14 weeks, oral	dog	<8.0	8.0	Effects on haematological parameters, clinical biochemistry, liver microsomal enzymes, changes relative prostate weight and histopathological changes in the adrenal gland	H. Wetzig, E. Hartmann (2001)
Spirodiclofen (BAJ 2740)	52 weeks oral	dog	1.45	4.54	Increased adrenal weight and adrenal vacuolation	H. Wetzig, Chr. Rühl-Fehlert (2001)

In a 13 week oral toxicity study with mice, effects were observed on body weight, haematology and cholesterol in the highest dose group. Changed relative organ weights were observed for adrenals, spleen, kidneys, testes and ovaries in the mid and high dose groups. Histopathological examination showed hypertrophy and vacuolation of Leydig cells in the testes and in the adrenals cytoplasmic vacuolation, degeneration of cortical cells and mononuclear infiltrate in the (mid and) high dose group. The liver showed besides periportal cytoplasmic vacuolation in the high dose group also centrilobular hepatocellular hypertrophy in all dose groups. A NOAEL could not be established in this study, and the LOAEL is 15.3 mg/kg bw/day.

In a 14 week oral toxicity study with rats, substance-related effects were mainly observed in the two highest dose groups and included decreased bw, changes in haematological parameters, effects on clinical chemistry including AP, GLDH, cholesterol, triglycerides and changed organ weights of liver, spleen, adrenals, testes and thymus. Histopathological examination showed epithelial vacuolation in jejenum in the highest two dose groups, whereas adrenal cortical vacuolation accompanied by increased grading of this effect was observed ~~in all~~ in the highest three dose groups in females. Hence the NOAEL in this study is 100 ppm, equal to 8.1 mg/kg bw/day.~~A NOAEL could not be established in this study, and the LOAEL is 6.6 mg/kg bw/day.~~

Three oral toxicity studies with dogs were performed. In an 8 weeks study (males only) and a 14 weeks study, comparable effects were observed and included clinical biochemical parameters as ALAT, ASAT, AP cholesterol and triglycerides, induction of liver enzymes (ECOD, ALD in both studies, N-DEM, O-DEM P450 in the 14 week study) and changed organ weights of liver, prostate, thyroid and adrenals. Histopathological examination showed effects on liver, testes and adrenals. In both studies, a NOAEL could not be established, and the LOAELs are 2.9 mg/kg bw/day in the 8-week study and 8.0 mg/kg bw/day in the 14 week study.

In the 52 weeks oral toxicity study with dogs, several clinical biochemical parameters and relative and

absolute organ weights were affected in the two lower dose groups, mainly without dose-relationship (i.e. effects are more or less constant or decreasing over the exposure groups). In the absence of associated histopathological changes in these dose groups and the fact that histopathological changes are only observed for a few organs at much higher doses, the observed effects at the lower doses are considered not clearly adverse. The adrenals are regarded to be the critical target organ. At and above 150 ppm, increased adrenal weights were observed accompanied by increased vacuolation of the adrenals, an effect which was also observed in several other toxicological studies with different species. Based on the effects observed on the adrenals in the two highest dose groups, the NOAEL in this study is, in accordance with the opinion of the study author, 50 ppm, equal to 1.45 mg/kg bw/day.

B.6.5 LONG-TERM TOXICITY AND CARCINOGENICITY

In the Evaluation Table (open point 2.6) the MS are to confirm the relevant NOAEL for long-term studies at an expert meeting. Moreover, UK requested more transparency in the study summaries (reporting table 2(11)), and the notifier submitted some position papers. Furthermore RMS was requested to transfer information regarding effects in the chronic feeding study in rats, as provided in the reporting table at 2(33) to the addendum (open point 2(13)).

B.6.5.1 Chronic toxicity and carcinogenicity

STUDY 1 Oncogenicity study in mice

The notifier submitted a position paper, proposing a higher NOAEL than that proposed by the RMS. However, the RMS does not agree and the reaction of the RMS on the notifier's comments were already incorporated in the reporting table dated 04.03.2005, see2(32).

For transparancy, the notifier's comments, and the reaction of the RMS as reported in the reporting table are copied below.

Notifier: Whereas the RMS concludes that the NOAEL in this study is < 25 ppm, BCS considers this dose level to be tolerated without adverse effects. Only at 3500 ppm treatment-related changes were seen.

RMS: The NOAEL of < 25 ppm was based on an increased incidence of adrenal pigmentation and vacuolation in females, an increased incidence of amyloid in several tissues of males and increased incidence of hepatocytomegaly in males.

The notifier states that the observed increase in adrenal corticomedullary pigmentation was within the historical control range. However, since pigmentation increases with age, the provided historical control values of a 92 weeks exposure study are not comparable with the values in the present 78 weeks exposure study. The provided historical control values of a 81 weeks exposure study showed pigmentation in 12/50 females, which is comparable with

the control values in females of the present study. The observed increase in the 25 ppm female group (20/49) is therefore considered a substance related effect. The difference in frequency between the lowest dose and the two higher doses is considered to be related to the great jump in doses (25 ppm vs 3500 and 7000 ppm).

Furthermore, the notifier states that the observed incidence in adrenal cortical vacuolisation at 25 ppm in females was not increased over control levels and is a common finding. However, an increased vacuolation was noted in females of all dose groups. The difference in frequency between the lowest dose and the two higher doses is considered to be related to the great jump in doses (25 ppm vs 3500 and 7000 ppm).

In addition, the notifier states that there was no increase in the number of animals with amyloidosis, and that the amyloidosis noted in the study was not compound related.

However, increased incidence and/or increased average severity of amyloid was observed in several tissues in the exposed animals. Increased incidence of amyloid was already observed in the lowest dose group in the heart, liver, thyroids and parathyroids of males. The historical control values of a 79-81 weeks exposure study are substantially lower than the observed increase in the 25 ppm group.

The notifier additionally argued that the observed hepatocytomegaly at 25 ppm is a normal response and was not significantly increased over control.

However, although not statistically significant, an increase in hepatocytomegaly at 25 ppm was noted in males. As no historical control data were available in the study report no additional evaluation of this finding could be made.

Based on the above considerations, the RMS is of opinion that the NOAEL should be adapted based on the comments made by the notifier. The NOAEL is set at < 25 ppm.

STUDY 2

Questions were raised in the Evaluation Meeting on missing details in the study summary. Moreover, the notifier submitted a position paper, proposing a higher NOAEL than that proposed by the RMS. Therefore the study summary is copied from the DAR, and detailed information on the weights of thymus and ovary is added and highlighted. Moreover, in contrast to the DAR, the NOAEL for chronic, non-neoplastic effects is clearly specified in the summary below.

Characteristics

reference/notifier	:	Wirnitzer, U., U. Bach, E. Hartmann (2000)	Exposure	:	108 weeks (interim necropsy after 52 weeks), neurotoxicity tested at week 77
type of study	:	Combined study on chronic toxicity and carcinogenicity.	Doses[1]	:	0, 50, 100, 350, 2500 ppm (0, 2.04, 4.11, 14.72, 110.14 mg/kg bw/day for males and 0, 2.87, 5.93, 19.88, 152.90 mg/kg bw/day for females)
year of execution	:	1997/1998	Vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 98.6%, 98.5%, 97.9%, 97.8%, 97.6%, 97.8%)	GLP statement	:	Yes[2]
route	:	Oral (diet, with 1% peanut oil)	Guideline	:	In accordance with OECD 453, with some deviations (see study design)
species	:	Rat, Wistar (HSd Cpb:WU)	Acceptability	:	Acceptable, with the exception of neurotoxicity.
group size	:	50/sex/dose (additional animals for interim necropsy after 1 year 10/sex/dose)	**NOAEL**	:	5.93 mg/kg bw/day

1 Dose levels are based on the results of a 14 weeks study with rats. Check of the test compound content in the diet showed lower than the defined concentrations at 50 ppm (3 times observed) and 100 ppm (2 times observed).

2 two deviations were reported: one out of ten analytical contents check which was re-evaluated after the end of the in-life phase was found to be invalid due to insufficient documentation; determination of test substance concentration in plasma samples as well as experiments for method development (telomere measurements in different tissues) were not conducted according to GLP. According to the study author, results are reported separately, but are not included for the present evaluation.

Study design

The study was in accordance with OECD guideline 453, with the following deviations: no high dose satellite group (20/sex) and satellite control group (10/sex) for evaluation of pathology other than neoplasia were present; blood samples for hematological examination should be collected from 20 rats/sex of all groups; neurotoxicity was tested on week 77 (Functional Observational Battery) with the observer aware of the animal's treatment assignment and the test runs were done in the same order (control-50 ppm-100 ppm- 350 ppm- 2500 ppm). Results of this 77 weeks neurotoxicity study are presented as a chronic neurotoxicity study in section B.6.7.3 of the DAR.

Results

The results of the study are summarized in table 6.5.1.2. Details on several organ weights at terminal sacrifice are presented in tables 6.5.1.2a and 6.5.1.2b.

Table 6.5.1.2

Dose (ppm)	**0**		**50**		**100**		**350**		**2500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No toxicologically relevant effects										
Clinical signs	No toxicologically relevant effects										
Body weight (gain)[1]									ds	ds	
Food consumption (rel)									i	i	

Dose (ppm)	0		50		100		350		2500		dr
	m	f	m	f	m	f	m	f	m	f	
Ophthalmoscopy											
-% eyes with post capsular lens opacity	21.9		24.2		24.7		39.4		31.7		
Haematology[2]											
-leukocytes									d	ds	
-lymphocytes									d	ds	
Urinalysis	No toxicologically relevant effects										
Clinical chemistry											
-AP									is	is	
-cholesterol									d	d	
-triglycerides									d	d	
-urea[3]										d	
-T4[4]									is		
-TSH[5]									i	is	
-P[6]									d	d	
Organ weights (12 months sacrifice)											
- brain										is[r]	
- adrenals[7]									i[r]		
- heart									is[r]	ds[a]	
- spleen									d[a,r]	ds[a]	
- thymus									i[r]	is[r]	
- testes									i[r]		
Organ weights (24 months sacrifice)											
- adrenals			i[a,r]		i[a,r]		i[a,r]		i[a,r]		
- liver									ds[a]		
- spleen				ds[r]		d[r]		ds[r]	d[a]	d[a]/ds[r]	
- thymus								i[a,r]	i[a, r]	i[a,r]	f
- testes									i[r]		
-ovaries								i[a,r]		i[a,r]	f
Pathology (12 months sacrifice)											
macroscopy	No toxicologically relevant effects										
Microscopy											
nonneoplastic lesions											
Liver											
-fat content single cells, cell clusters and periportal hepatocytes									d		
Eyes											
Degeneration/ retinal	2/10		0/10		0/10		0/10		5/10		
Adrenals											
- cytopl. vacuolation (Zona fasciculata), small											
Incidence	6/10		7/10		5/10		3/10		9/10		
average grading	1.3		1.0		1.0		1.0		2.2		
- cytopl. vacuolation (Zona fasciculata), large											
Incidence	6/10		5/10		4/10		5/10		10/10		
average grading	1.5		1.4		1.3		1.2		1.5		
- adrenocorticocellular hypertrophy											
Incidence	0/10		0/10		0/10		0/10		3/10		
average grading	0		0		0		0		1.7		
Spleen											
- hemosiderin decomposition											
mean severity	3.1	3.9	3.3	3.8	3.5	3.5	2.9	3.9	1.9	2.9	
Thyroid											
- C-cell hyperplasia	0/10		0/10		0/10		0/10		4/10		

Dose (ppm)	0		50		100		350		2500		dr
	m	f	m	f	m	f	m	f	m	f	
Pathology (24 months sacrifice)											
macroscopy											
Liver											
- discoloration (intercurrent deaths)		3/21		4/18		0/19		1/15		8/24	
Microscopy											
nonneoplastic lesions											
Eyes											
- lenticular degeneration	5/31		11/30		is 14/36		is 13/31		11/41		
- retinal atrophy (deaths only)		7/21		7/18		7/19		5/15		13/24	
Jejenum											
- vacuolated enterocytes	3/31	2/29	0/30	0/32	4/36	0/31	3/31	0/35	is 18/41	is 14/26	
Adrenals											
-diffuse hypertrophy/vaculation cortex cells (Z. fasc.)	3/31		1/30		5/36		4/31		is 25/41		
- focal hypertrophy (Z. fasc.) (deaths only)	1/19		0/20		0/14		0/19		2/9		
Nasal cavity											
- atrophy.degeneration olfactory epithelium	8/31		14/30		12/36		11/31		is 25/41		
Thyroid											
-colloidal alteration	23/50		23/50		28/50		28/50		35/50		
- follicular cell hyperplasia		1/29		0/32		1/31		0/35		5/26	
Liver											
- tigroid basophilic focus	10/31		8/30		12/36		9/31		19/41		
- cholangiofibrosis	9/31		5/30		11/36		5/31		18/41		
- focal necrosis (deaths only)		0/21		2/18		1/19		1/15		is 5/24	
Testes											
- focal Leydig cell hyperplasia	4/31		4/30		4/36		6/31		is 19/41		
Microscopy											
neoplastic lesions[8]											
Testes											
- benign Leydig cell tumor (except deaths)	2/31		1/30		0/36		4/31		9/41		
- benign Leydig cell tumor (deaths only)	0/19		0/20		0/14		0/19		1/9		
Uterus											
- adenocarcinoma (except deaths)		2/29		3/32		2/31		0/35		3/26	
- adenocarcinoma (deaths only)		2/21		2/18		1/19		2/15		is 11/24	
Thyroid											
- C-cell adenoma (except deaths)		1/29		2/32		3/31		4/35		4/26	
- C-cell adenoma (deaths only)		1/21		0/18		0/19		1/15		2/24	

dr dose related
ds/is statistically significantly decreased/increased
d/i decreased/increased
a/r absolute/relative organ weight

1 The difference was significant nearly throughout the whole treatment period in males (up to 11%) and from week up to and including week 53 in females (up to 8%). (Mean individual water intake was slightly lower at 2500 ppm in both sexes).

2 The RMS does not subscribe the opinion of the study author, that the observed significantly decreased leukocyte and lymphocyte counts in females are mainly due to relative high control values, and thus not considered to be of toxicological relevance, since decreased lymphocyte and leukocyte numbers were observed at almost all time points in males and females, though not statistically significant.

3 Observed at weeks 79 and 105.

4 Significantly increased at weeks 53 and 105, increased at weeks 27 and 79.

5 Males: observed at weeks 53, 79 and 105; females: increased at week 53, significantly increased at weeks 79 and 105.

6 Males: significantly decreased at weeks 27 and 53, decreased at weeks 79 and 105; females: significantly decreased at weeks 27 and 79, decreased at weeks 53 and 105.

7 Mean absolute adrenal weights in males were 50 and 54 mg (interim sacrifice) in controls and highest dose group respectively. Taken into account that bw was significantly decreased in the highest dose group, the observed increase in adrenal weight is expected to be more pronounced considered relative to body weight. However, due to the presented relative organ weights in round numbers by the study author, the expected increased rel adrenal weights were not observed. In view of the present evaluator, organs with low weights should not be presented in round numbers. Rough calculations with the mean values resulted in rel. adrenal weights of 9.54 and 11.49 in males of control and highest dose group respectively, which is considered an increase in rel adrenal weight.

8 In females of the highest dose group that had died intercurrently, metastasis of carcinoma were observed in several organs, including spinal cord, forestomach, glandular stomach, liver, pancreas, kidneys, ovaries, lymph nodes, spleen femur, body cavities.

Table 6.5.1.2a Thymus and ovary weights at terminal sacrifice in grams ± SD (and % increase compared to control)

Dose (ppm)	0	50	100	350	2500
Thymus (males)					
- absolute (mg)	200 ± 49.9	227 ± 72.4 (14%)	220 ± 70.5 (10%)	207 ± 67.4 (4%)	229 ± 66.1 (15%)
- relative (mg/100 g bw)	38 ± 8.1	44 ± 13.7 (16%)	40 ± 12.0 (5%)	40 ± 12.7 (5%)	46 ± 11.9 (21%)
Thymus (females)					
- absolute (mg)	172 ± 45.5	188 ± 59.3 (9%)	192 ± 83.2 (12%)	205 ± 129.4 (19%)	215 ± 70.4 (25%)
- relative (mg/100 g bw)	56 ± 15.3	58 ± 17.0 (4%)	59 ± 23.2 (5%)	62 ± 36.6 (12%)	67 ± 19.1 (20%)
Ovaries					
- absolute (mg)	153 ± 70.3	164 ± 76.9 (7%)	147 ± 32.2 (-4%)	172 ± 103.9 (12%)	206 ± 135.3 (34%)
- relative (mg/100 g bw)	49 ± 22.6	51 ± 22.2 (4%)	45 ± 11.3 (-8%)	55 ± 42.9 (12%)	64 ± 40.7 (31%)

Table 6.5.1.2b Adrenal and spleen weights at terminal sacrifice in grams and % increase compared to control

Adrenals (males)									
- absolute (mg)	65	77	18%	82	26%	73	12%	82	26%
- relative (mg/100 g bw)	13	16	23%	16	23%	14	8%	16	23%
Adrenals (females)									
- absolute (mg)	79	84	6%	80	1%	86	9%	75	-5%
- relative (mg/100 g bw)	26	26	0%	25	-4%	27	4%	24	-8%
Spleen (males)									
- absolute (mg)	1131	1130	0%	1191	5%	1129	0%	1016	-10%
- relative (mg/100 g bw)	217	222	2%	225	4%	220	1%	207	-5%
Spleen (females)									
- absolute (mg)	702	642	-9%	696	-1%	649	-8%	625	-11%
- relative (mg/100 g bw)	226	199	-12%	213	-6%	199	-12%	196	-13%

Acceptability

The study is considered acceptable for the evaluation of of long term toxicity and carcinogenic potential of the substance. Since a Functional Observational Battery was conducted in week 77, this part of the study is not considered in this 2 year study, but separately as a neurotoxicity study.

Conclusions

Effects on body weight, food consumption, haematology and clinical chemistry were observed in the highest dose group only. After 12 months of exposure, changed organ weights were only observed in the highest dose group. After 24 months of exposure, increased adrenal weights (m) and decreased spleen weights (f) were observed in all dose groups. These changed organ weights were not dose-related and not accompanied by histopathological observations in the lowest dose groups. Other observations in the lowest dose groups were lenticular degeneration of the eyes and degeneration of olfactory epithelium of the nasal cavity, which were both not dose-related. Total observed thyroid C-cell adenomas were increased in the 350 and 2500 ppm dose groups, but based on historical control data not relevant. In these two highest dose groups, increased weights of thymus and ovaries were observed. Since in females the absolute and relative weights of thymus and ovaries were increased by > 10%, this effects is considered to be test substance related. Hence the NOAEL for chronic toxicity is set at 100 ppm, equivalent to 5.93 mg/kg bw/d.

Spirodiclofen is considered carcinogenic for inducing Leydig cell tumors and uterus adenocarcinomas in the rat. These tumors were observed at and above 110.14 mg/kg bw/day, the NOAEL for neoplastic lesions is therefore 14.72 mg/kg bw/day.

STUDY 3

1-year oral toxicity study in dogs

Unfortunately, this study is summarised in the DAR in section B.6.5.1 Chronic toxicity and carcinogenicity. However, a 1-year study in dogs is considered semi-chronic, and is in this addendum included in B.6.3.

After the EPCO meeting a developmental neurotoxicity (DNT) study was evaluated, and is presented in B.6.7.4 of this revised addendum.

B.6.6 REPRODUCTIVE TOXICITY

In the Evaluation Table (open point 2.7) the MS are to confirm the relevant NOAEL for reproduction toxicity studies at an expert meeting. Moreover, UK requested more transparency in the study summaries (reporting table 2(11)), and the notifier submitted a position paper.

B.6.6.1 Reproductive toxicity

STUDY 1

Questions were raised in the Evaluation Meeting on missing details in the study summary. Therefore the study summary is copied from the DAR, and detailed information on critical effects is added and highlighted.

Characteristics

reference/notifier	:	R. Eiben (2000)	Exposure	:	F0: 12 weeks pretreatment F0; F1 offspring untill weaning of F2.
type of study	:	Two-generation study.	Doses[1]	:	0, 70, 350, 1750 ppm (F0: 5.2, 26.2, 134.8 mg/kg bw/day for males and 5.5, 27.6, 139.2 mg/kg bw/day for females; F1: 6.4, 30.2, 177.6 mg/kg bw/day for males and 7.0, 34.4, 192.7 mg/kg bw/day for females)
year of execution	:	1997-1999	Vehicle	:	-
test substance	:	BAJ 2740 (spirodiclofen, purity 98.6%)	GLP statement	:	Yes
route	:	Oral (diet)	Guideline	:	OECD 416
species	:	Wistar rat (Crl: WI (WU) BR)	Acceptability	:	Acceptable
group size	:	25/sex/dose	**$NOAEL_{syst}$**	:	< 5.2 mg/kg bw/day
			$NOAEL_{repr}$		26.2 mg/kg bw/day

1 dose selection was based on the results of a one-generation pilot study with dietary administration of 0, 250, 2500 and 1000 ppm spirodiclofen, with an overall NOAEL of 250 ppm.

Study design

The study was performed in accordance with OECD guideline 416. The test substance was administered to parental (P) animals prior to and during their mating, during the resultant pregnancy and through the weaning of their F1 offspring. The substance was then administered to selected F1 offspring during their growth into adulthood, mating, and production of a F2 generation, until weaning of the F2 generation.

During lactation period of food intake was not determined.

Parameters of reproduction in F0 parents included length of estrus cycle (determined about 3 weeks before mating of F0), insemination index, fertility index, gestation index and duration of pregnancy, and in males of dose groups 0 and 1750 ppm: sperm morphology, sperm motility, sperm count epididymides and spermatid head count in testis.

Results

The results of the study are presented in table 6.6.1.1. Details on organ weights of the F0 generation, and clinical chemistry of the F1 generation at terminal sacrifice are presented in table 6.6.1.1a.

Table 6.6.1.1

Dose (ppm)	**0**		**70**		**350**		**1750**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	None								
Clinical signs	No toxicologically relevant effects								
Body weight[1]			d		ds	d	ds	ds	m,f
Food consumption	No toxicologically relevant effects								
Mating/fertility/gestation	No toxicologically relevant effects								
Pathology									
Organ weight									
- brain			is^r		is^r		is^r	$is^{r,a}$	

Dose (ppm)	0		70		350		1750		dr
	m	f	m	f	m	f	m	f	
- adrenals							is[r]	is[r]	
- liver			ds[a,r]		ds[a,r]		ds[a,r]		
- kidneys								ds[a, r]	
- testes							is[r]		
- prostate			is[r]		is[r]				
- epididymides (left)			ds[r]		ds[r]				
- seminar vesicles			ds[r]		d				
Pathology									
macroscopy	No toxicologically relevant effects								
microscopy									
Small intestine									
- epithelial vacuolation	4/25	6/25	0/25	7/25	5/25	5/25	17/25	10/25	
Adrenal glands									
- mean severity vacuolation	2.0	1.2	1.9	1.2	1.8	1.6	2.7	2.1	
Testes									
- diminished in size			1/25		1/25		4/25		
Epididymides									
- diminished in size			1/25		1/25		4/25		
F1 pups									
Litter size	No toxicologically relevant effects								
Survival index	No toxicologically relevant effects								
Sex ratio	No toxicologically relevant effects								
Body weight									
- at birth			i			ds		ds	
- during lactation					ds	ds	ds	ds	m, f
Organ weights F1 weanlings									
- brain					i[r]	i[r]	i[r]	i[r]	m,f
- spleen					d[r]		d[r]		m
Pathology									
macroscopy	No toxicologically relevant effects								
F1 animals									
Mortality	No toxicologically relevant effects								
Clinical signs	No toxicologically relevant effects								
Body weight					d		ds	ds[3]	m
Food consumption							is	is	
Mating/fertility/gestation									
- spermatids per mg testis			nd		-1%		-23%		
- sperms per mg epididymides			nd		+10%		-18%		
Clinical chemistry									
- AP							is	is	
- Cholesterol			d		ds		ds	ds	m
- Triglycerides			ds	d	ds	d	ds	ds	m, f
- UFA (unesterified fatty acids)					d	d	ds	ds	m, f
- Creatinine						i		is	f
Organ weight									
- brain							is[a, r]		
- adrenals							is[a, r]	is[a, r]	
- liver							ds[a, r]		

Dose (ppm)	0		70		350		1750		dr
	m	f	m	f	m	f	m	f	
- kidneys								ds[r]	
- ovaries								i[a, r]	
- uterus								i[a, r]	
Pathology									
macroscopy	No toxicologically relevant effects								
microscopy									
Testes									
- atrophy, diffuse	0/25		1/25		1/25		4/25		
Epididymides									
- oligospermia	0/25		1/25		1/25		4/25		
- atrophy	0/25		1/25		1/25		4/25		
Adrenal glands									
- vacuolation, mean severity	1.9	1.2	2.0	1.2	2.1	1.5	2.8	2.1	m,f
Prostate									
- atrophy	0/25		0/25		0/25		3/25		
Small intestine									
- epithelial vacuolation		10/25		12/25		13/25		17/25	f
Ovaries									
- vacuolation, mean severity		1.4		1.3		1.3		1.8	
F2 pups									
Litter size	No toxicologically relevant effects								
Survival index	No toxicologically relevant effects								
Sex ratio	No toxicologically relevant effects								
Body weight									
- at birth					ds	ds	ds	ds	m, f
- during lactation							ds	ds	
Organ weight[4]									
- spleen								d[r]	
- thymus			d[r]		d[r]	d[r]	d[r]	d[r]	f
Pathology									
macroscopy	No toxicologically relevant effects								
microscopy	Not performed								

dr dose related

1 mean bw of females of dose group 70 ppm in week 0 was significantly higher compared to controls (about 6%), and mean bw of females of dose group 350 ppm was about 2% higher compared to mean control bw weight.

2 Mean pup weight at birth and during lactation (day 0 – day 28)

3 Significantly decreased bw from week 17 to 21 (lactation period)

4 Determined in 5/sex/dose group; no statistics performed.

Body weights of male F0 animals receiving 70 ppm were statistically significantly decreased during weeks 1 to 6, however, the deviations were below 5%. At 350 ppm statistically significant lower body weights were observed at several times with a maximum of –5.1% in weeks 12-15. In 1750 ppm males there was a statistically significant (-7%) retardation in body weights. In F0 females body weights were not affected up to 350 ppm. At 1750 ppm statistically significant lower body weights occurred mainly during the lactation period (-15.2% in week 19). In F1 animals, body weights of males and females receiving 70 or 350 ppm were not affected. At 1750 ppm decreased body weights were noted in males (up to –23% in week 1) and females during the lactation period (-10.3 on day 14).

Table 6.6.1.1a Details on organ weights of F0 generation, and clinical chemistry of F1 generation at terminal sacrifice

Dose (ppm)	0		70		350		1750	
	m	f	m	f	m	f	m	f
Organ weights								
Terminal body weight (g)	498	260	481	266	476	254	463	235
Liver								
- absolute (g)	18,826	11,842	16,605**	12,461	16,535**	11,944	15,991**	10,214**
			-12%	5%	-12%	1%	-15%	-14%
- relative (mg/100g BW)	3,78	4,55	3,45**	4,68	3,47**	4,70	3,45**	4,35
			-9%	3%	-8%%	3%	-9%	-4%
Clinical chemistry								
Cholesterol (mmol/l)	2.55	2.24	2.30	2.33	2.07**	2.20	1.58**	1.69**
	±0.229	±0.373	±0.366	±0.362	±0.236	±0.268	±0.261	±0.307
Triglyceriden (mmol/l)	2.62	1.36	1.84**	1.22	1.68**	1.02	0.96**	0.58**
	±0.667	±0.461	±0.469	±0.551	±0.712	±0.354	±0.193	±0.166

According to the study report, the values of CHOL en TRIGL were within the ranges of historical control data during 1996-1997. However, only minimal details were provided (ranges were indicated, no mean values). Therefore these values cannot be properly assessed.

Acceptability

The study was considered acceptable.

Conclusions

Body weight in F0 animals was dose-relatedly decreased in males of all dose groups and in females at and above 350 ppm. In males of all dose groups a significantly decreased liver weight (not dr) and significantly increased brain weight was observed. In females dosed at and above 350 ppm adrenal gland vacuolation severity was increased.

In F1 pups, a significantly decreased body weight at birth and during lactation was observed at and above 350 ppm (dr). In F1 weanlings decreased relative spleen weights in males (dr) and increased brain weights (m/f, dose-related) at and above 350 ppm were observed.

F1 animals showed decreased blood cholesterol (10-40%, m) and triglyceride (30-70%) concentrations (dr) at all dose levels. In the high dose group, decreases were observed in the number of spermatids in the testes and the number of sperms in the epididymides, and weights of brain, adrenals, liver, kidneys, ovaries and uterus had changed.

The F2 pups showed significantly decreased body weight at birth at and above 350 ppm (dr) and also during lactation decreased body weight of F2 pups was observed in the highest dose group (-17% in males and –21% in females). In females dosed at and above 350 ppm a dose-related decreased thymus weight was observed.

Based on the observed effects in the lowest dose group in the F0 and F1 animals, a NOAEL could not be established in this study, and the LOAEL for systemic toxicity is 70 ppm, equal to 5.2 mg/kg

bw/day.

Based on the decreased spermatogenesis in the F1 males the NOAEL for reproduction is 350 ppm, equal to 26.2 mg/kg bw/d.

B.6.7 NEUROTOXICITY

B.6.7.4 Developmental neurotoxicity

The notifier informed the RMS that a developmental neurotoxicity (DNT) study was included in the EU dossier on the occasion of the 2004 update, but never formally submitted to the RMS. At the completion of the addendum in June 2005, the study was not yet received by the RMS, and could therefore not be included.

After the EPCO meeting the submitted DNT study was evaluated.

Developmental neurotoxicity study in rats

Characteristics of study:

Reference	:	Sheets *et al.*, 2004	Route	:	oral, diet
Year of execution	:	2002	Group size	:	30 females/dose
Guideline	:	OPPTS 870.6300 (EPA, 1998)	Exposure	:	gestation day (GD) 0 to lactation day (LD) 21 (dams) to post-natal day 21 (offspring)
GLP	:	yes	Doses	:	0, 70, 350, 1500 mg/kg food[1]
Test substance	:	Spirodiclofen, batch no. 06480/0002; beige powder	Vehicle	:	diet
Purity	:	97%	Acceptable	:	yes
Species	:	rat, Wistar Hannover Crl:WI (Glx/BRL/Han) IGS BR	**NOAEL**mat	:	350 mg/kg food (32 mg/kg bw/d)
			NOAELdev	:	350 mg/kg food (32 mg/kg bw/d)
			neurotoxic effects	:	not observed

[1] equal to 0, 6.5, 32, 136 mg/kg bw/d (gestation) and 0, 14, 70 and 274 mg/kg bw/d (lactation)

Study design

The study was performed in accordance with EPA guideline OPPTS 870.6300.

In short, the study was executed as follows:

Animals were exposed as described above. On post-natal day (PND) 4, litters with a minimum of eight pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 20 litters per level, were subjected to evaluation using the following observations and measurements: preputial separation or vaginal patency, body weight, food consumption, a functional observational battery (FOB), automated measures of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance after weaning and a water maze task on PND 60) and an ophthalmic examination. Serum cholesterol was measured in the dams (LD 21) and offspring (PND 4 and 21) and neural tissues were collected from 10 rats/sex/dietary level (representing approximately 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and

morphometry.

The following modifications of the guideline were implemented:

- Increased number of offspring for morphometric and neuropathological evaluation (10/sex/dose instead of 6)
- Extended exposure (GD 0 to PND21 instead of GD 6 to PND 10).
- Animals were perfused before collecting brains at the end of exposure on PND 21 and 75.

The first two changes increased the sensitivity of the study, while the last increased the quality of the samples. Therefore, these changes with respect to the guidelines are considered acceptable.

Results

The results are presented in Table 6.7.4.1, 6.7.4.2 and 6.7.4.3.

Table 6.7.4.1 Overview of results for dams and litter data

Dose (mg/kg food)	**0**	**70**	**350**	**1500**	**dr**
F-0 dams					
Mortality	0/30	1/30	0/30	0/30	
Clinical signs		no treatment related findings			
Pregnant animals	29	29	29	30	
Body weight					
GD 0-20		no treatment related findings			
LD 0-14		no treatment related findings			
LD 14-21			d (4%)	dc (5%)	
Food consumption					
GD 0-6				dc (16%)	
GD 6-20		no treatment related findings			
LD 0-7		no treatment related findings			
LD 7-14			d (6%)	dc (8%)	
LD 14-21		no treatment related findings			
FOB		no treatment related findings			
Organ weight					
Pathology					
macroscopy		no treatment related findings			
Litter data					
Life foetuses		no treatment related findings			
Viability index		no treatment related findings			
Lactation index		no treatment related findings			
Pup weight					
PND 0-11		no treatment related findings			
PND 17			d (5%)	dc (5%)	
PND 21			d (4%)	dc (8%)	yes
Pup weight gain			d (5%)	dc (9%)	yes

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

Table 6.7.4.2 Overview of results for F-1

Dose (mg/kg food)	0		70		350		1500		dr
	m	f	m	f	m	f	m	f	
F-1 animals									
Clinical signs	no treatment related findings								
Body weight	no treatment related findings								
Food consumption	no treatment related findings								
Sexual maturation	no treatment related findings								
Pupil constriction (PND 21)	no treatment related findings								
Ophthalmoscopy	no treatment related findings								
FOB	no treatment related findings								
Motor and locomotor activities	no treatment related findings								
Acoustic startle	no treatment related findings								
Passive avoidance	no treatment related findings								
Water maze									
Learning phase	no treatment related findings								
Retention phase - trials to criterion[1]				ic (47%)		ic (26%)		i (53%)	
Clinical chemistry	no treatment related findings								
Brain weight	no treatment related findings								
Pathology									
macroscopy	no treatment related findings								
microscopic measurements	m	f	m	f	m	f	m	f	
- caudate putamen size PND-21			ne	ne	ne	ne		dc (3%)	
- caudate putamen PND-75			ne	ne	ne	ne		ic (3%)	
- parietal cortex PND-75			ne	ne	ne	ne	dc (6%)		
histopathology	no treatment related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
ne not examined
[1] tested for significant differences between control group and individual treated groups with Fisher's exact test

Table 6.7.4.3 Trials to criterion in water maze test for female rats in retention phase

Dose (mg/kg food)	0	70	350	1500
Number of Animals	16	16	16	16
Trials to Criterion (Mean + S.D.)	5.8 ± 1.9	8.5 ± 3.7*	7.3 ± 2.3*	8.9 ± 4.5

p < 0.05 in with Fisher's exact test

Acceptable

The study is considered acceptable.

Conclusions

The water maze test showed a significantly decreased memory performance for low and mid dose females. In high dose females, this performance was also less than in the control group, but the difference was not statistically significant. No clear dose relation was observed and the dispersion of the measurements was quite high, varying between ca. 30 to 50%, increasing the likelihood of false positive results. Therefore, it is doubtful that the test substance had a negative influence on memory performance in the water maze test. Furthermore, according to a re-analysis of the study results made by a third party (de Raat and Verhaar (2005)), not linked to the laboratory that executed the study) in assignment of the notifier the number of trials to criterion is an inadequate measure of learning and retention, as it disregards the amount of errors made before reaching the criterion (five consecutive errorless trials). Instead de Raat and Verhaar performed analyses of covariance using the variables "number of errors to criterion", "dose" and "sex". The outcome of these analyses showed that treatment did not influence the number of errors made, be it in the learning or retention phase of male or female rats. The RMS agrees with this approach and the conclusions it yielded.

Microscopic brain sections of high dose and control groups of the offspring were measured at PND-21 and PND-75. At PND-21, in females, the caudate putamen was decreased in size, while at PND-75 it was increased. At PND-75 the parietal cortex size was decreased in males. All these changes were relatively small (between 3 and 6%). Furthermore, no histopathological changes were observed in the brain areas concerned. Therefore, these changes in brain measurements are not considered of toxicological significance.

Only in the high dose group (1500 mg/kg food), statistically significant, but minor, effects were observed both in the dams as in the offspring. In the dams these effects were limited to occasional decreases in food consumption in the first days of gestation and midway lactation, which only in the last week of lactation led to decreased body weight, be it minor (5%). In the offspring, pup weight (gain) was reduced. Based on these effects, the NOAEL for maternal and developmental effects is set at 350 mg/kg food (equal to 32 mg/kg bw/d; LOAEL 136 mg/kg bw/d).

Note by the RMS:

Bayer also submitted this DNT study to the US-EPA and Canada's PMRA for an application for the registration of spirodiclofen in the USA and Canada. EPA and PMRA concluded that the compound showed an effect on memory retention in rats as measured in a water maze test, hence no NOAEL could be determined. Moreover, it was concluded that a treatment-related change in brain morphometric parameters occurred in the rats subjected to this test. These conclusions were taken into account during the evaluation of the DNT study by the RMS.

B.6.8 FURTHER TOXICOLOGICAL STUDIES

In the Evaluation Table (open points 2.2 and 2.8) the MS are to discuss the endocrine disrupting properties and carcinogenic effects of the compound.

B.6.8.1.2 Mechanistic studies

The notifer submitted a position paper (2.2) comprising an expert report on the possible endocrine-mediated toxicity of spirodiclofen, which is summarised below.

STUDY 1 Sittert N.J. et al (2002).

As the mechanism of observed effects of spirodiclofen in adrenals, testis and uterus, the notifier proposed that the compound indirectly interferes with steroid hormone synthesis via an effect on the generation of co-substrate NADPH (so called 'reducing equivalents').

The authors of the report indicated that cholesterol is the precursor for both gonadal and adrenal steroid hormone biosynthesis. The bulk of the cholesterol is derived by uptake from plasma cholesterol (esterified) rather than by intracellular synthesis. The first step in the steroidogenesis involves cleavage of the terminal six carbons of the side chain of cholesterol, resulting in the formation of pregnenolone. From pregnenolone a number of pathways lead to the formation of metabolic intermediates that give ultimately rise to the synthesis of androgenic and estrogenic sex hormones. Conversion of cholesterol to pregnenolone occurs within the mitochondria. Enzymes involved in this step are collectively known as cholesterol side-chain cleavage cytochrome P-450 complex, comprising various NADPH-dependent hydroxylases. Pregnenolone is then released from the mitochondria in the smooth endoplasmatic reticulum and biotransformed in progesterone, by the enzyme 38-hydroxysteroid dehydrogenase-A4'5 -isomerase. Another dehydrogenase involved in synthesis of testosterone is 178-hydroxysteroid dehydrogenase. Microsomal cytochrome P-450-dependent monooxygenases involved in testosterone synthesis are NADPH-dependent 17a-hydroxylase (or steroid-17a-monooxygenase) and C-17,20-lyase (17a-hydroxyprogesterone aidoiase). Inhibition by spirodiclofen or its metabolites of one or more of these enzymes would be a critical event leading to decreased levels of testosterone, progesterone or 178-estradiol.

To function, several of these enzymes need co-substrate NADPH. Thus interference with the

formation of mitochondrial and cytoplasmatic NADPH by spirodiclofen or its metabolites would also have consequences for steroid hormone biosynthesis. Cytoplasmatic NADPH is also required for cholesterol and fatty acid synthesis and inhibition may result in lower blood levels of these substances.

The authors evaluated essentially the same studies as described in the DAR and addendum. Important target organs following subchronic and chronic dosing of spirodiclofen to mice, rats and dogs are the adrenal glands and the testes. It was shown that spirodiclofen produces:

• generalised enlargement of these organs in mice, rats and dogs;
• vacuolation of adrenal cortex in mice, rats and dogs;
• increased size of testicular cells (hypertrophy) in mice, rats and dogs;
• increase in testicular cell number (hyperplasia) in mice and rats;
• testicular and uterine tumours in the rat.

As both the adrenal glands and the gonads produce and release steroid hormones in response to adrenocorticotrophic and gonadotrophic hormones released from the pituitary, it is expected that the effects of spirodiclofen on adrenal glands and gonads are produced through an endocrine-mediated mechanism.

The authors concluded that the two-generation reproduction study in rats and developmental toxicity studies in rats and rabbits did not show effects on fertility and reproduction parameters, such as estrus cycle, insemination index, fertility, sperm count and production, livebirth index, viability index, lactation index and uterus weight. Neither were developmental effects observed. The NOAEL for reproductive toxicity was based on reduced parental and pup body weights. The results thus indicated that the influence of spirodiclofen on the endocrine tissues, if any, was apparently weak. In agreement with the notifier, the authors concluded that according to EPA criteria, spirodiclofen has no androgenic or estrogenic properties in mammals.

> **NOTE by the RMS**: *in the DAR and addendum, the NOAEL for reproductive toxicity is based on the decreased spermatogenesis in the high dose F1 males (NOAEL 26.2 mg/kg bw/d, LOAEL 134.8 mg/kg bw/d).*

Indications of endocrine-mediated effects were increased LH levels in plasma of dogs and decreased estradiol and progesterone levels in plasma of rats in subchronic studies. Based on the data base, including the mechanistic studies, the authors support the notifier's conclusion that spirodiclofen interferes with the generation of NADPH, which is an important co-substrate in several steps of the biosynthesis of steroid hormones. However, several assumptions had to be made which were not experimentally verified, e.g. quantitative data of reduced NADPH levels were not available.

In summary, according to the authors, the adrenal, testicular and uterine effects are the result of the following mechanism. A high dietary level of spirodiclofen increases the release of adrenocorticotrophins and gonadotrophins from the pituitary as a result of spirodiclofen-induced inhibition of steroid hormone biosynthesis. The increase in gonadotrophins then results in chronic stimulation of testicular Leydig cells and endometrial uterine cells, resulting in hypertrophy,

hyperplasia and tumour formation. Spirodiclofen was not mutagenic/genotoxic or clastogenic in *in vitro* and *in vivo* test systems, which confirms that the spirodiclofen-induced carcinogenic response in rats is through a hormone-mediated non-genotoxic mechanism for which a threshold dose exists, below which no carcinogenic effects occur.
Spirodiclofen-induced testicular and uterine carcinogenicity could not be demonstrated in mice and dogs, which suggests a species-specificity for the rat. It is unlikely that this rat-specificity results from species differences in spirodiclofen-inhibition of steroid hormone synthesis, because there is no reason to assume that BAJ 2510-inhibition of malate dehydrogenase is qualitatively and quantitatively different among species. Species differences may, therefore, result from differences in gonadotropin-mediated effects.
With regard to the hepatocellular adenomas and carcinomas found in mice in the high-dose groups, the authors regard it to be unlikely that these tumours were induced through an endocrine-mediated mechanism. In analogy with observations in dogs, at high dose levels spirodiclofen may induce certain drug metabolising cytochrome P450-dependent enzymes in mouse liver. Liver enzyme induction in the mouse might subsequently result in hypertrophy, hyperplasia and tumour formation, as also shown for organochlorine pesticides. Tumour induction by organochlorine pesticides was not observed in other species, including humans. Spirodiclofen-induced liver tumours in mice are, therefore, deemed as mouse-specific and not of relevance to humans.

The question whether or not spirodiclofen should be considered as an endocrine active compound (EAC) cannot be answered with a simple yes or no. Endocrine active compounds (also referred to as endocrine disrupters) can be defined as chemical substances that modify the normal functioning of human hormone systems or that alter hormonal regulation. Biological endpoints to be associated with EAC's are, for example, reproduction and developmental parameters, steroid receptor binding or inhibition, altered hormone levels, altered cell proliferation and cell differentiation, etc. The authors feel that the nature and severity of the hormonal effect should also be taken into account for classification of chemicals as EAC.
Hormone-mediated effects of spirodiclofen include vacuolation, hypertrophy, hyperplasia, testicular and uterine cancer. Furthermore, spirodiclofen induced an increase in LH release from the pituitary observed in male dogs and a decrease in levels of progesterone and estradiol in female rats, which are also seen as hormonal effects. On the other hand, spirodiclofen does not act via an androgen or estrogen-receptor mediated mechanism and spirodiclofen or its metabolite BAJ 2510 do not interact with enzymes involved in hormone steroid biosynthesis. Most importantly, the two-generation toxicity study in rats and developmental toxicity studies in rats and rabbits did not show effects on fertilty, reproduction and developmental parameters at levels which did not cause parental toxicity. The primary effect of spirodiclofen is disturbance of NADPH generation in mitochondria and cytoplasma through inhibition of malate dehydrogenase. This triggers a cascade of hormone-mediated events.
The authors feel that, according to the above definition, spirodiclofen is an endocrine active compound. However, it should be placed in the same category as, for instance, ethanol, which compound acts through a similar mechanism *(e.g.* through NADPH depletion).

Reaction of the RMS: the above mentioned mechanism seems plausible and we agree with the conclusions of the authors.

B.6.8.1.3 Toxicological assessment of the new proposed specification

The notifier reported the following (text in italic):

Introduction

The dossier of spirodiclofen for the application for inclusion in Annex I of 91/414/EEC was submitted to the EU in July 2001. The description of the production pathway of spirodiclofen, the analytical results of representative batches and the specification documented in this dossier refer to a pilot plant production of the active substance. Meanwhile the final chemical production process has been established. This production pathway differs in some details from the pilot plant production process because two of the former intermediates are now commercially available. The present document describes the new production pathway, summarizes the analytical results of 5 batches hereof, and presents a modified specification including a toxicological and ecotoxicological assessment of the new specification.

Purity of the active substance

The specified concentration of spirodiclofen (purity) was increased from min. 955 g/kg to min. 965 g/kg. This is acceptable from a toxicological perspective as it will have no significant influence on the toxicity of spirodiclofen technical.

Concentration shifts for existing impurities

The concentration of BAJ 2740-enol remained unchanged at max. [redacted] g/kg whereas [redacted] and [redacted] could be reduced to max [redacted] g/kg. This is acceptable from a toxicological perspective.

New impurities

Three new impurities were identified in the 5 batch analysis: [redacted] (no. 05), N,N-dimethylacetamide (no. 06) and [redacted] (no. 07). The toxicological relevance of these impurities is assessed below.

[redacted] has been tested for its acute oral toxicity in rats and for the induction of point mutations in S. typhimurium (No critical results were obtained; the LD50 is > 2500 mg/kg bw and no induction of point mutation was seen). Thus, a concentration of maximal [redacted] g/kg is toxicologically acceptable for this impurity.

***N,N-dimethylacetamide** is a well known chemical which has been under broad use as a solvent since decades and a comprehensive package of toxicological data is available for this chemical. Its toxicological properties are comparable to that of other strong solvents: - The*

chemical is of moderate acute toxicity following inhalation or dermal exposure. - The liver is the main target after repeated exposure (inhalatory, dermal, oral). - Developmental effects are seen after exposure of pregnant animals to high dose levels.

The official classification of N,N-dimethylacetamide in the European Union is Repro 2 / R61; Xn / R 20/21.

A workplace threshold limit value has been set in the USA and in Germany at 10 ppm (36 mg/m³). When taking this value (which is equivalent to a systemic dose of ~ 5 mg/kg bw/day) for a comparison to a worst case exposure to N,N-dimethylacetamide from the use and han-dling of a spirodiclofen formulation, a high margin of safety results: At a specified concentra-tion of [redacted] % of N,N-dimethylacetamide in spirodiclofen techn., the estimated worst-case ex-posure of an operator who is exposed to 100% of the acceptable operator exposure (AOEL) set for spirodiclofen (0.009 mg/kg bw/day) to N,N-dimethylacetamide is 0.0000[redacted] mg/kg bw/day. This is by a factor of >100 000 below the tolerable exposure at workplaces. Thus, for N,N-dimethylacetamide a specified limit of max. [redacted] g/kg can be supported from a toxicological perspective.

[redacted] is a main [redacted] following [redacted] of spirodi-clofen [redacted] Thus, this compound can be regarded as covered by the toxicological studies performed with the parent molecule and the specified limit of max. [redacted] g/kg is toxico-logically acceptable.

Comments by the RMS:

The argumentation provided by the notifier on the toxicological equivalence of the new production process is considered acceptable. The MSDSs of [redacted] N,N-dimethylacetamide, and [redacted], and the reports of the Ames study (Herbold, 2002) and acute oral toxicity rat study (Schüngel, 2002) on [redacted] were submitted, and the two studies are summarised below.

Ames test, plate incorporation/preincubation method

Study design and results

In an initial test doses of 16, 50, 158, 500, 1581 and 5000 µg/plate were used. However, only doses up to 50 µg/plate did not cause bacteriotoxic effects and precipitation occurred at the dose of 1581 µg/plate. No mutagenic activity was obnserved in this intial study. The study was repeated using lower dose levels, see below:

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.* TA 98 TA 100 TA 102 TA 1535 TA 1537	point mut. point mut. point mut. point mut. point mut	- - - - -	- - - - -	rat liver	Arochlor 1254	25, 50, 100, 200, 400, 800, 1600 µg/plate solvent: DMSO	Herbold, 2002
Test substance: [redacted] batch no. [redacted], purity [redacted] %, white powder Cytotoxicity observed at dose level: 200 µg/plate							

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Precipitation observed at dose level: - GLP statement: yes According to OECD 471: yes							

Acceptability
The study was considered acceptable.
Conclusions
The test substance did not induce point mutations in *S. typhimurium*.

Acute oral toxicity
Characteristics

reference	Schüngel, 2002	exposure	once by gavage
type of study	acute oral toxicity study	doses	2000 mg/kg bw
year of execution	2002	vehicle	2% Cremophor EL in water
test substance	[redacted], batch no. M [redacted], purity [redacted]%, white powder	GLP statement	yes
route	oral	guideline	in accordance with OECD 423
species	rats, Wistar HsdCpb:Wu	acceptability	acceptable
group size	3/sex	LD_{50}	> 2500 mg/kg bw

Study design
The study was performed in accordance with OECD 423.
Results
Mortality: one female died within 5 h after administration.
Symptoms of toxicity: Diarrhoea and increased water intake were observed up in all males up to day 5; abdominal position, decreased motility, laboured breathing, narrowed palpebral fissure, reactivity decreased, uncoordinated gait were observed in the females on day one only.
Body weight: no treatment-related findings
Pathology: in the femaale that died pale discoloured liver and kidneys, dark-red discoloured spleen, and a slightly collapsed lung were observed.
Acceptability
The study is considered acceptable.
Conclusions
The acute oral LD_{50} of the test substance was estimated to be >2500 mg/kg bw for rats.

For the new impurity [redacted], not only a (negative) Ames test (as required by the Guidance Document (Sanco/10597/2003-rev7 final 2, 14 dec 2005) but also an acute oral toxicity test was submitted (LD50 > 2500 mg/kg bw). These two tests did not indicate a changed toxicity profile of spirodiclofen. Eventhough the second new impurity **N,N-dimethylacetamide** is toxic for reproduction, the provided calculation based on inhalation exposure, indicates that a sufficient safety margin is present even if dermal or oral exposure is considered. The third new impurity [redacted] **acid** is not mentioned in the [redacted], but the molecular structure indicate that indeed it is an [redacted] following [redacted]

B.6.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL AND DRINKING WATER LIMIT

In the Evaluation Table (open points 2.9 and 2.10) the MS are to confirm the ADI and AOEL at an expert meeting.

B.6.10.3 ADI and AOEL

For the setting of the ADI and AOEL it is proposed to use the NOAEL of 1.45 mg/kg bw/d from the 1-year dog study. Unfortunately, the 1-year dog study was included in B.6.5 (Chronic studies), however, a 1-year dog study is considered semi-chronic, and should have been included in B.6.3. As there seems to be no effect of exposure duration (based on studies in rat, mice and dogs), the NOAEL of 1.45 mg/kg bw/d is considered to be applicable for both short-term and chronic exposure. Application of a safety factor for inter- and intraspecies differences of 100 results in an **ADI** of 0.0145, rounded to 0.015 mg/kg bw/day.

For establishment of an internal **AOEL**, a safety factor of 100 is used and for correction of incomplete oral absorption a factor of ~~0.64~~ 0.65 is used. This results in an internal systemic short-term AOEL of 0.009 mg/kg bw/day, rounded to 0.009 mg/kg bw/d, equal to 0.63 ~~0.65~~ mg/person/day for a 70 kg person, and 0.54 for a 60 kg person.

B.6.12 DERMAL ABSORPTION

Dermal absorption of ^{14}C-BAJ 2740 in male rhesus monkeys.

During and after the expert meeting, the dermal absorption study was considered in more detail, hence some corrections and additions were made to the original summary and evaluation that was presented in the DAR. The corrected version is presented below. Only the changes compared to the original evaluation presented in the DAR are highlighted.

Characteristics

Reference	:	Z. Wu (2002)	exposure	:	single dose (occlusion)
type of study	:	Absorption, distribution, excretion and metabolism	doses	:	151 µg/animal
year of execution	:	2002	vehicle	:	BAJ2740 Blank suspension in water
test substance	:	[Dihydrofuranone-3-^{14}C]BAJ2740 (spirodiclofen) (radiochemical purity 99%)	GLP statement	:	yes
Route	:	dermal	guideline	:	US-EPA 870.7600
Species	:	Rhesus Monkey	acceptability	:	Acceptable
group size	:	5 males			

Study design

The sponsor provided a ready-to-use formulation BAJ2740 SC 240 containing radiolabeled ^{14}C BAJ 2740 (1.51 µg/µL).

Five naïve male rhesus monkeys received a dermal application, under occlusion, of 100 µL of the test substance, containing in total 151 µg ^{14}C-BAJ2740, to the shaven skin (4 cm x 6 cm). Subsequently, the animals were restrained in a primate chair for 8 h and then placed in metabolism cages. At 8 h after dosing the patch was removed, the application site was washed with cotton swabs dipped in soapy water. Next the application site was tape-stripped 16 times, and wiped with isopropyl alcohol swabs and soapy water swabs. Urine, faeces and samples of cage rinse and the final cage wash were collected up to 144 h post dosing. Following removal from the primate chair samples of chair wash were collected. All samples were analyzed for radioactive content.

Results

At 8 h after application 84.53 % of radioactivity was recovered in the dermal washes, with 58.75% being recovered in the first 4 cotton swabs. In tape strips and isopropylalcohol swabs 0.11 and 1.46 % were recovered respectively. From the securing materials and application site patch 2.19 and 1.93 % of radioactivity were recovered respectively. In urine, faeces, cage rinse/wash and chair wash a total of 2.12% (range 1.31 – 3.48%) of administered radioactivity was recovered over 144 h, with about 1.7% being excreted within the first 24 h. Total recovery of radioactivity was 92.34 %.

Table 6.12.1 Total recoveries of BAJ 2740-dihydrofuranone-3-[^{14}C]-derived radioactivity at 144 hours following dermal administration of BAJ 2740 SC 240 containing ^{14}C BAJ 2740 to male Rhesus monkeys at a target dose of 151 µg/animal

Recovery	Sample	Percentage of dose (%)						
		Animal number					Mean	SD
		1001	1002	1003	1004	1005		
Elimination	Urine	1.56	1.98	1.21	1.92	0.50		
	Faeces	0.00	0.07	0.06	0.29	0.08		
	Cage debris/rinse	0.08	1.33	0.00	0.24	0.79		
	Chair/urine pan wash/wipe	0.12	0.1	0.04	0.24	0.00		
	Cage wash/wipe	0.00	0.00	0.00	0.00	0.00		
	Subtotal	**1.76**	**3.48**	**1.31**	**2.69**	**1.37**	**2.12**	**0.94**
Residual	Patch/securing material	4.89	5.96	4.14	4.52	1.08		
	Swabs	81.43	80.72	89.61	84.98	93.21		
	Tape strips	0.08	0.15	0.04	0.14	0.13		
	Subtotal	**86.40**	**86.83**	**93.79**	**89.64**	**94.42**	**90.22**	**3.77**
Total		**88.16**	**90.13**	**95.10**	**92.33**	**95.79**	**92.34**	**3.21**

In some animals elimination still occurred in the last study period (120-144 hours after application): 0.02% in urine and 0.02% in feces in one animal, 0.14% in faeces in one animal, and 0.10% in cage debris/rinse in one animal.

Table 6.12.2 Elimination of BAJ 2740-dihydrofuranone-3-[^{14}C]-derived radioactivity by male Rhesus monkeys following dermal administration of BAJ 2740 SC 240 containing ^{14}C BAJ 2740 at a target dose of 151 µg/animal

Sample	Time (hours)	Percentage of dose (%)						
		Animal number					Mean	SD
		1001	1002	1003	1004	1005		
Urine	0-4	0.15	0.19	0.00	0.19	0.02		
	4-8	0.60	NS	0.29	0.81	NS		
	8-12	0.55	1.16	0.52	0.46	0.00		
	12-24	0.26	0.30	0.30	0.25	0.34		
	24-48	0.00	0.15	0.10	0.11	0.14		
	48-72	0.00	0.08	0.00	0.08	0.00		
	72-96	0.00	0.02	0.00	0.00	0.00		
	96-120	0.00	0.06	0.00	0.02	0.00		
	120-144	0.00	0.02	0.00	0.00	0.00		
	Subtotal	**1.56**	**1.98**	**1.21**	**1.92**	**0.50**	**1.43**	**0.61**
Faeces	0-4	NS	NS	NS	NS	NS		
	4-8	0.00	0.00	NS	0.00	NS		
	8-12	0.00	NS	NS	NS	NS		
	12-24	0.00	0.00	0.02	0.03	0.00		
	24-48	0.00	0.00	0.04	0.10	0.07		

	48-72	0.00	0.05	0.00	0.00	0.01		
	72-96	0.00	0.00	0.00	0.00	0.00		
	96-120	0.00	0.00	0.00	0.02	0.00		
	120-144	0.00	0.02	0.00	0.14	0.00		
	Subtotal	**0.00**	**0.07**	**0.06**	**0.29**	**0.08**	**0.10**	**0.11**
Cage debris/ rinse	8-12	0.00	0.22	0.00	0.24	0.69		
	12-24	0.00	0.32	0.00	0.00	0.00		
	24-48	0.08	0.00	0.00	0.00	0.00		
	48-72	0.00	0.00	0.00	0.00	0.00		
	72-96	0.00	0.00	0.00	0.00	0.00		
	96-120	0.00	0.79	0.00	0.00	0.00		
	120-144	0.00	0.00	0.00	0.00	0.10		
	Subtotal	**0.08**	**1.33**	**0.00**	**0.24**	**0.79**	**0.49**	**0.56**

Acceptability

In the EPCO meeting, experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. A number of member experts indicated that they would not have accepted the study initially, particularly as there were clear OECD guidelines on both in vivo and in vitro assessment of dermal absorption. Areas of concern with the monkey study included the fact that levels of radioactivity in the skin and body were not determined, and the level of total radioactivity recovered (92%). The low level of variation in individual animals supported the theory that the 8% of radioactivity lost may have been absorbed, and thus the experts concluded that this should be incorporated into the dermal absorption to give a value of 10%.

~~It is noted that about 8% of administered radiolabel was not recovered. This could represent radiolabel located in skin or in the body. Since excretion of radioactivity towards the end of the study period was low, it can be assumed that, whatever amount is located in the skin, will only be absorbed to a small extent. These data suggest that complete excretion of absorbed radioactivity occurred in this study. Therefore, the dermal of radioactivity (2.12%) can be normalized to 100% total recovery, resulting in a normalized dermal absorption of 2.3%.~~

Conclusion

~~The in vivo dermal absorption of BAJ2740 in rhesus monkeys is approximately 2%. It is proposed to use this estimate for extrapolation to human dermal absorption of BAJ 2740.~~
It was concluded during the EPCO expert meeting that the in vivo dermal absorption of BAJ2710 in rhesus monkeys is approximately 2+8=10%.

Overall conclusion on dermal absorption

During the EPCO meeting, experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. Hence experts

considered the physical chemical properties of spirodiclofen, and considered that the molecular weight and K_{OW} supported a dermal absorption value of 10%. It was therefore concluded that the dermal absorption be set at a value of 10% based on physicochemical properties and supported by the studies in monkeys.

It was additionally noted that no data was available on the dermal absorption potential of the formulation dilution. Therefore a value of 65% was proposed, based on the oral absorption value.

However, a re-assessment by the RMS after the EPCO meeting revealed that these conclusions were drawn on wrong assumptions. According to the GAP the concentrated formulation contains 240 g as/L, and the spray dilution is 0.048-0.096 g as/L. In the in vivo study a dose of 1.51 g/L was tested, which is a factor of over 150 lower than the concentrate and a factor of 16 to 32 higher than the spray dilution. Moreover, the area dose applied to the monkeys was 151 µg/24 cm^2 = 6.3 µg/cm^2. This rather low area dose is an acceptable area dose to be used for the spray concentration. Hence the RMS now proposes a dermal absorption value of 10% for the concentrate and spray dilution. This is also more in line with the general practice of applying one default value for both the concentrate and the spray dilution.

The applicant still is of the opinion that a dermal absorption for concentrate and spray dilution of ca 2% is justified. They claim that the results of an exploratory study in monkeys indicated an overall recovery of 108.3%. Moreover, the total recovery of the i.v. part of that study (107.27%) indicated that the administrered dose was not retained in the body. According to the RMS, this exploratory study turns out to be a rather important study, however, it was not submitted in the EU-dossier, hence the study itself could not be evaluated. The short summary provided in the conclusion of the main study, and some more details that the applicant made available to the RMS after the EPCO meeting (not peer-reviewed), indeed indicate that a dermal absorption value of ca 2% might be more realistic. Since at this stage this study cannot be accepted anymore in the Annex-I procedure, the RMS proposes that the acceptability of the exploratory study, and a possible lowering of the proposed dermal absorption of 10% to ca. 2% will be a Member State issue after the Annex I inclusion.

B.6.14 EXPOSURE DATA

Based on the revised dermal absorption value, the exposure scenarios and the risk assessment were recalculated.

B.6.14.1 Calculations

External operator exposure values without and with personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM). These calculations are presented in appendix 1.

For estimation of worker exposures during re-entry activities (e.g. harvesting fruit) no formally accepted predictive model or database has yet been developed. Nevertheless, a general algorithm for the calculation of worker exposure is widely accepted. This algorithm is based on the concept of 'dislodgeable foliar residue'.
For bystander exposure during manual or mechanical downward spraying, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for bystanders. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment, therefore the use of this equipment is not considered in bystander risk assessment.

Both the calculations of bystander exposure and worker exposure are presented in appendix 1.

Dermal absorption of spirodiclofen in humans when exposed to the undiluted and diluted formulation was estimated to be 10%, based on physicaochemical properties of spirodiclofen, and the *in vivo* monkey data described in section B.6.12. For inhalation exposure a default value of 100% was used.

The basic assumptions, input data and calculations used in the risk assessment for the use of spirodiclofen are further specified in Appendix 1.

B.6.14.2 Exposure and risk assessment

Inetrnal exposures and risks as % AOEL are specified in Table 6.14.2.1, 6.14.2.2 and 6.14.2.3.

Table 6.14.2.1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic[1] (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic[1] (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying in grapes						
UK- 75th	Respiratory	0.029	0.029	0.54	5	5
	Dermal	2.60	0.96	0.54	481	178
	Total exposure	**2.63**	**0.99**	**0.54**	**487**	**183**
DE- GM	Respiratory	0.01	0.01	0.63	2	2
	Dermal	1.07	0.09	0.63	169	14
	Total exposure	**1.08**	**0.10**	**0.63**	**172**	**16**
Mechanical upward spraying in pome fruits, and stone fruits						
UK- 75th	Respiratory	0.03	0.03	0.54	5	5
	Dermal	3.32	1.03	0.54	615	191
	Total exposure	**3.35**	**1.06**	**0.54**	**621**	**197**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	1.60	0.13	0.63	254	20
	Total exposure	**1.62**	**0.15**	**0.63**	**258**	**24**
Mechanical upward spraying in citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	3	3
	Dermal	2.74	0.62	0.54	508	116
	Total exposure	**2.76**	**0.64**	**0.54**	**510**	**118**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	1.60	0.13	0.63	254	20
	Total exposure	**1.62**	**0.15**	**0.63**	**258**	**24**
Manual upward spraying in grapes						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2
	Dermal	7.46	1.12	0.54	1381	208
	Total exposure	**7.47**	**1.13**	**0.54**	**1383**	**210**
DE- GM	Respiratory	0.02	0.02	0.63	2	2
	Dermal	1.06	0.03	0.63	168	4
	Total exposure	**1.08**	**0.04**	**0.63**	**171**	**6**
Manual upward spraying in pome fruits, and stone fruits						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2
	Dermal	7.46	1.12	0.54	1381	208

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic[1] (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
	Total exposure	**7.47**	**1.13**	**0.54**	**1383**	**210**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	1.59	0.04	0.63	252	6
	Total exposure	**1.61**	**0.06**	**0.63**	**256**	**10**
Manual upward spraying in citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	1	1
	Dermal	6.97	0.89	0.54	1291	164
	Total exposure	**6.98**	**0.89**	**0.54**	**1292**	**165**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	1.59	0.04	0.63	252	6
	Total exposure	**1.61**	**0.06**	**0.63**	**256**	**10**

[1] The AOEL systemic is 0.54 mg/day, using a body weight of 60 kg for the UK-model, and 0.63 mg/day, using a body weight of 70 kg for the German model.

Table 6.14.2.2 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic (mg a.s./day)	%AOEL
Exposure during mechanical upward spraying on pome/stone fruit, citrus, and grapes			
Respiratory	<0.01	0.63	<1
Dermal	0.43	0.63	68
Total exposure	**0.44**	**0.63**	**70**

Table 6.14.2.3 Worker internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic (mg a.s./day)	% AOEL	
	without PPE	with PPE		without PPE	with PPE
Exposure after mechanical upward spraying on apples and pears					
Respiratory	-	-	-	-	-
Dermal	1.9	0.19	0.63	302	30
Total exposure	1.9	0.19	0.63	302	30

- The inhalation exposure is not quantifiable with this method.

Conclusions

- Safe uses by operators with PPE were identified for mechanical and manual upward spraying in pome fruits, stone fruits, citrus and grapes using the German model. No safe uses were identified using the UK model.
- Safe use for bystanders was identified for mechanical and manual upward spraying in pome fruits, stone fruits, citrus and grapes using the EUROPOEM II 2002 model (90th percentile).
- Safe use for workers with PPE was identified for mechanical and manual upward spraying in pome fruits, stone fruits, citrus and grapes using the EUROPOEM II 2002 model (90th percentile).

B.6.15 REFERENCES

Only references additional to those mentioned in the DAR are mentioned here.

References for the active substance

Annex point / reference no.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA, 5.3.2.1/ 02	Hartmann, E.	2005	Update on historical control data, adreno-cortical vacuolation subchronic rat studies – status January 2005. Bayer AG, Report No.: unknown Date: 28 January 2005 No GLP, unpublished	Y	BAY
IIA, 5.8.1.2.3.2/ 21	Van Sittert N.J., J. Krüse, C.N. Groeneveld	2002	The possible endocrine effects of BAJ 2740: A critical evaluation. Generated by: OpdenKamp Registration & Notification Document: BA\END-01.1845/4 OAG document: 01001845.WPD Source: Bayer CropScience Date : 29 May 2002 No GLP, Unpublished	Y	BAY
IIA, 5.8.2/01	Sheets, L.P., Lake, S.G.,	2004	A Developmental Neurotoxicity Screening Study with Technical Grade Spirodiclofen in Wistar Rats, [redacted], Report Number 201056, GLP: yes	Y	BAY
IIA, 5.8.2/02	Raat de, W.K., Verhaar, H.J.M.	2005	Evaluation of a developmental neurotoxicity study with spirodiclofen, ENVIRON, Project No: 77ba-spi	Y	BAY
	Herbold, B.	2002	BAJ-2740 Hexyl acid – Salmonella/microsome test, plate incorporation and preincubation method. Bayer Health Care, Report number AT00093, GLP: yes	Y	BAY
	Schüngel, M	2002	BAJ-2740 Hexyl acid – Acute toxicity in the rat after oral administration. [redacted], Report number AT00127, GLP: yes	Y	BAY

B.7 RESIDUE DATA

Introduction

Spirodiclofen is intended to be used as a acaricide in a concentration of 4.8 g a.i/hL (mandarins) or 9.6 g a.i./hL (pome fruit, stone fruit, orange, grape). The maximum dose level per hectare is 96 g a.i./ha for grape and 144 g a.i./ha for pome fruit, stone fruit and citrus. Spirodiclofen is applied as foliar application by means of a BAJ 2714 SC 240 suspension concentrate containing 240 g/L spirodiclofen.

The draft assessment report was sent to the member states by EFSA in April 2004. Member States and EFSA comments were gathered in a reporting table, which was commented by the notifier and the rapporteur. After the evaluation meeting in February 2005, 4 data requirements and 3 open points were pointed for further evaluation.
The data requirements:

1. extraction efficiency of incurred samples of grape extracted with Method 00568;
2. recoveries of determination of residues in apple pomace with Method 00568 at 1.0 mg/kg;
3. providing processing studies on apple and orange and concomitant evaluation;
4. providing more validation data for method of analysis for animal products (Method 109720).

Open points:

1. Rapporteur to perform MRL-calculations on data sets for pear South, apple South, and the whole pome fruit data set
2. discussion about the fat soluble parent and the not-fat soluble metabolite M01 in animal tissue (awaite expert meeting)
3. depending on discussion of open point 2, MRLs for animal products.

After discussion in the evaluation meeting in February 2005, an addendum was composed which was discussed in an Expert meeting EPCO 29, June 2005. This revised addendum contains the conclusions from the Evaluation Meeting and EPCO 29.

B.7.3 Residue definition

B.7.3.2 Definition of the residue in animal products

In female goat, spirodiclofen was metabolised and the parent compound was not found. The major metabolic product in all tissues and milk was the hydrolysed derivative M01 (80.7%-95.4% TRR per tissue or milk). The oxygenated derivative of spirodiclofen-enol (M03) was

also present in milk, liver and kidney but at lower levels. The metabolite M01 is therefore considered to be a good marker for tissues and milk.

In additionial feeding studies on lactating cows with 13.1 mg/kg dw feed, parent was found in milk fat (cream) and in fat and in another cow, M01 was found in kidney. At lower dose rates (1.29-3.93 mg/kg dw feed) no residues (parent or M01) were found in any of the tissues nor in the milk. The theoretical maximum exposure for ruminants is 0.38 mg/kg dw feed/d (corresponding to 0.016 mg/kg bw/d).

The goat metabolism study indicated that no parent compound was detected with the metabolite M01 being detected at the highest levels (> 80%TRR) in all matrices (study conducted at >600N rate). The cow feeding studies were therefore conducted at 34x – 10x – 3.4x the theoretical exposure rate. The female cow feeding studies at the 34x dose indicate that parent should also be part of the residue definition in animal commodities. Metabolite M01 is not fat soluble whereas parent compound is. However, since parent compound was only found in milk cream at the highest dose rate (13.1 mg/kg dry feed) in the feeding study with cow and the uses considered in the DAR do not give rise to significant residue in animal products it was concluded in the expert meeting (EPCO 29, d.d. June 2005) that M01 only is suitable for the residue definition.
If the use is extended to other crops fed to animals and the animal intakes significantly increase then the residue definition will need to be re-considered at a MS level.

Definition of the residue for enforcement and risk assessment in animal products:
Because fruit pomace is fed to ruminants and the trigger value of 0.1 mg ai/kg dw feed for cattle is exceeded, a residue definition for animal products is needed and an MRL should be set. Because M01 is the main component in the female goat study and parent was only found in the female cow feeding studies at a 10N dose level, the following definition of the residue of animal products is proposed:

spirodiclofen-enol, expressed as spirodiclofen.

B.7.6 Residues resulting from supervised trials

Extraction efficiency of method of analysis M0568 (plants)

STUDY 1

Characteristics

reference	:	Robin Sur, 2004 (6.2.1/01)	GLP statement	:	yes
type of study	:	Method of analysis	guideline	:	-
year of execution	:	2004	species	:	grape
test substance	:	[dihydrofuranone-3-^{14}C]-spirodiclofen	route	:	Foliar application

Study design

A sample from the previously evaluated study of Babczinski and Bornatsch (1999, DAR), investigating the metabolism of spirodiclofen on grapes, was used for doing an additional experiment on extraction efficiency of Method 00568. Grapes were treated by foliar application with 0.22 kg [dihydrofuranone-3-^{14}C]-spirodiclofen. Samples of grapes were harvested after 3 weeks and kept frozen at –20°C till analysis.

Duplicate samples were extracted with acetonitrile/water according to Method 00568. Total radioactive residue was determined for the extracted as well as the non-extracted residue (solids). Identification and quantification was performed by radio-TLC.

Results

Total radioactive residues as well as parent compound were determined and compared with the extraction efficiency of Method 00568 as is shown in Table B.7.2.1

Table B.7.2.1: Total radioactive residue and ^{14}C-spirodiclofen in grape samples from study of Babczinski and Bornatsch (1999) (methanol/water extraction, 2nd column), and extracted with acetonitrile/water (Method 00568 as used in the residue trials, 3rd column).

sample:	methanol/water		acetonitrile/water	
	%TRR	Mg eq/kg	%TRR	Mg eq/kg
TRR	100	1.83	100	1.90
Total extracted	97.4	1.78	99.9	1.90
spirodiclofen	93.0	1.70	96.4	1.84
Post-extraction solids	2.3	0.05	0.1	0.00
Extraction efficiency = 1.78/1/83 * 100 = 97%			**spirodicolfen = 1.78/1.83 * 100 = 93%**	

In the draft monograph it was concluded that the intended use for grapes is 0.096 kg ai/ha with 1000 L/ha water and a PHI of 14 d. The metabolism study is performed at a 2.3x exaggerated dose, both with a very long pre harvest interval (64 d) and a slightly longer pre harvest interval (21 d). The level found in the late application metabolism study (1.83 mg/kg

spirodiclofen in the extracts, divided by 2.3 = 0.80 mg/kg at PHI=21, assuming linear extrapolation) is much higher than the levels found in the supervised trials for grapes (0.020-0.091 mg/kg at PHI=21, see table B.7.6.3.5a/b, DAR).

Therefore it was hypothised that extraction efficiency with dichloromethane, methanol and methanol/water is much higher than with acetonitrile/water as used in the grape residue trials. The newly provided data unequivocally proves that extraction efficiency is similar for both methods.

Conclusions

The ^{14}C-labelled sample extracted with dichloromethane, methanol and methanol/water on the one hand and acetonitrile/water on the other hand showed similar results. Extraction efficiency of Method 00568 for spirodiclofen residue on grape is acceptable**.**

Furthermore, since the original sample was frozen for 7 years and spirodiclofen accounted for 93% of TRR, it was concluded that sample stability was at least 7 years in grape.

Guidelines and limitations

1. Identification was performed based on TLC –analysis only. Validation of TLC-results was not shown.
2. However, total radioactive residue extracted accounted for 97% with both extraction methods, which was high enough to assume that nearly all residue is extracted independent of the solvent/method. Therefore, the question raised about extraction efficiency was properly answered.

Validation of the residue analytical Method 00568 for apple pomace

STUDY 1

Characteristics

reference	:	Zimmer, D Gnielka, A (6.2.1/02)	GLP statement	:	yes
type of study	:	Method of analysis	guideline	:	-
year of execution	:	2004	species	:	Apple
test substance	:	spirodiclofen	acceptable	:	acceptable

Study design

Recoveries of spirodiclofen residue (parent only) were determined in various matrices after extraction and by determintation according to Method 00568 based on acetonitril/water extractions. Residues were quantified by a reversed phase HPLC-column coupled to a turbo-ionspray MS/MS detection.

The method validation took place by conducting recovery experiments for apple pomace.

This study was performed as an additional experiment to study 99/351/99, to show recoveries in apple pomace at a level of 1.0 mg/kg spirodiclofen.

Results

Recoveries of spirodiclofen in apple pomace were already determined at the 0.02 and 0.2 mg/kg level before in study 99/351/99. For completeness, these values are pointed in the table below again, together with the newly determined values spiked at 1.0 mg/kg.

Table B. 7.3.2 Recoveries of spirodiclofen after extraction in apple pomace

matrix	fortification level (mg/kg)	recoveries	mean ± rsd
Appe pomace	0.02	95, 85, 81	87 ± 8.3
	0.2	106, 111, 109	109 ± 2.3
	1.0	90, 98, 95	94 ± 3.1

Conclusion

Apple pomace samples spiked at 1.0 mg/kg showed good recoveries > 70% with relative standard deviation < 20%.

Guidelines and limitations

none

B.7.7 Effects of industrial processing and/or household preparations

B.7.7.1 Processing

STUDY 1

Characteristics

reference	:	Krolski, M.E. (6.2.1/02)	GLP statement	:	yes
type of study	:	Processing	guideline	:	Directive 91/414/EC; 7030/VI/95 rev.3, Appendix E
year of execution	:	2000	species	:	orange
test substance	:	spirodiclofen	acceptable	:	acceptable

Study design

Orange trees were treated once with foliar spraying at a dose level of 1.23 kg a.i./ha, which corresponds to a 9N dose. Orange fruit was harvested 7 DAT and separated into peel and fruit. Samples were kept frozen until further analysis. Samples were processed in a blender in dry ice within 27 days into juice, concentrated juice, dried pulp and orange oil. Extraction of spirodiclofen residue took place by a method similar to method M 00568 (acetonitrile/water extraction followed by hexane partitioning for separating the oil). Extracts were measured within 4 days. Determination took place by LC-MS/MS detection in which the LC was coupled to MS/MS detector by an electrospray interface.

Since the analytical method was similar to Method 00568 it is assumed valid for the processed fractions from 0.02 – 0.2 mg/kg, as was shown in the study of Nusslein (1999, DAR)). However, recoveries were determined in samples fortified at 0.01 and 0.1 mg/kg, and linearity of the detector response was determined at 0.002-0.100 ppm.

Dry matter percentage of dried pulp was determined by an IR-200 Moisture Analyser after drying at 100°C.

Results

Table B. 7.4.1.1 Recoveries of spirodiclofen after extraction in different orange fractions

Matrix	fortification level (mg/kg)	recoveries	mean ± rsd
Whole fruit	0.010	108, 106, 116	110 ± 5.3
	0.10	108, 106, 99	104 ± 4.7
	1.50	103, 104, 107	105 ± 2.1
Peel	0.010	108, 100, 98	102 ± 5.3
	0.10	97, 97, 107	100 ± 5.8
	1.50	79, 84, 86	83 ± 3.6
Peeled fruit	0.010	91, 89, 88	92 ± 5.1
	0.10	103, 94, 98	98 ± 4.5
Juice	0.010	106, 107, 106	106 ± 0.6
	0.10	112, 111, 107	110 ± 2.6
Concentrated juice	0.010	98, 100, 98	99 ± 1.1
	0.10	101, 100, 104	102 ± 2.1
	1.5	101, 109, 103	104 ± 4.2
Dried pulp	0.010	84, 95, 77	85 ± 9.1
	0.10	84, 90, 88	87 ± 3.1
	2.0	78, 76, 97	83.7 ± 11.6
Oil	0.010	79, 80, 97	85.3 ± 10.1
	0.10	111, 70, 80	87 ± 21.4
	100	78, 87, 85	83.3 ± 4.7

Table B. 7.4.1.2 Determination of spirodiclofen after extraction in different orange fractions

Matrix	measured residue (mg/kg)	mean ± rsd	concentration factor (CF)
Whole fruit	1.06, 1.12, 0.96	1.05	n.a.
Peel	1.24, 1.30, 1.27	1.27	1.2
Peeled fruit	0.07, 0.06, 0.05	0.06	0.06
Juice	0.05, 0.05, 0.05	0.05	0.05
Concentrated juice	0.151, 0.148, 0.139	0.15	0.14
Dried pulp	1.48, 1.38, 1.34	1.40	1.3
Oil	76.9, 73.1, 68.5	72.2	69

Linearity

The linearity of the detector response was > 0.996 for oil (0.004-0.100ppm) and > 0.998 (0.0002-0.100 ppm) (others).

Recovery

Recoveries were all > 70% with relative standard deviations < 20%, except for citrus oil at a level of 0.1 mg/kg. However, since recoveries in citrus oil at 0.01 mg/kg and 100 mg/kg were within the acceptable range, and overall recovery was 85.2 ± 12.2% it is concluded that the method is also suitable for determination of spirodiclofen in citrus oil.

Conclusion

The method used was validated (recoveries/linearity) for the different fractions and considered acceptable. Residue is associated more with peel and dried pulp (CF > 1) than with peeled fruit, juice and concentrated juice (CF < 1).

Guidelines and limitations

~~1. Only parent spirodiclofen was measured. Since hydrolysis during heating might result in considerable amounts of M01 (up to 50%), the study is not appropriate for deriving processing factors for doing risk assessments.~~

STUDY 2

Characteristics

reference	:	Harbin, A.M (6.2.1/02)	GLP statement	:	yes
type of study	:	Processing	guideline	:	Directive 91/414/EC; 7030/VI/95 rev.3, Appendix E
year of execution	:	2002	species	:	apple
test substance[1]	:	spirodiclofen	acceptable	:	acceptable

Study design

Apple trees were treated once with a foliar dose level of 1.28 kg a.i./ha, which corresponds to a 9N dose. Apple fruit was harvested 7 DAT. Apples were transferred to the laboratory within 4 hours and stored at 0°C for 7 days. A subsample was frozen at –5°C as blanc/100% value.

Processing took place by washing and concomitant pressing of the fruits in an apple press to separate juice from wet pulp. The juice was heated, cooled and depectinated to mimic commercial processing.

A second sample was peeled, cored, chopped, steamed and screened to produce apple coarse sauce. Moisture content and sweetness were adjusted, and the coarse sauce was heated until 155°C to produce the final apple sauce.

A third sample was peeled, cored, trimmed, sliced and dipped in a sulphite solution, whereafter it was dried in hot air to produce dried fruits.

Extraction of the processed commodities took place in acetonitrile/water (with 20% cysteine hydrochloride). After vacuum filtration the extracts were acidified with HCl and passed through a ENVI-carb solid phase extraction filter which was washed with acetonitrile/water and methanol, after which elution took place by methylene chloride. Analysis took place by LC-MS/MS.

Since the analytical method was similar to M 00568 it is assumed valid for the processed fractions from 0.02 – 0.2 mg/kg, as was shown in the study of Nusslein (1999). However, recoveries were determined in samples fortified at 0.01 and 0.1 mg/kg, and linearity of the detector response was determined at 0.002-0.100 ppm.

Dry matter percentage of dried pulp was determined by a Denver IR-200 Moisture Analyser after drying at 100°C.

Results

Table B. 7.4.1.3 Recoveries of spirodiclofen after extraction in different apple fractions

Matrix	fortification level (mg/kg)	recoveries	mean ± rsd
whole fruit unwashed	0.01	100, 94, 103	99 ± 4.6
	0.60	90, 87, 75	84 ± 7.9
whole fruit washed	0.01	91, 97, 100	96 ± 4.6
	0.50	84, 79, 84	82 ± 2.9
wet pomace	0.01	96, 98, 100	98 ± 2.0
	3.00	106, 112, 110	109 ± 3.1
Juice	0.01	111, 97, 98	102 ± 7.8
concentrated juice	0.01	116, 103, 105	108 ± 7.0
apple sauce	0.01	103, 105, 109, 100	104 ± 3.8
dried fruit	0.010	94, 102, 100	98.7 ± 4.2

Table B. 7.4.1.4 Determination of spirodiclofen after extraction in different apple fractions

Matrix	measured residue (mg/kg)	mean	concentration factor (CF)
whole fruit unwashed	0.575, 0.496, 0.588	0.55	n.a.
whole fruit washed	0.348, 0,387, 0,357	0.36	0.65
wet pomace	2.09, 2.02, 2.32	2.14	3.9
Juice	<0.01, <0.01, <0.01	<0.01	0.02
concentrated juice	<0.01, <0.01, <0.01	<0.01	0.02
apple sauce	<0.01, <0.01, <0.01, <0.01, <0.01	<0.01	0.02
dried fruit	<0.01, <0.01, <0.01	<0.01	0.02

Linearity

The linearity of the detector response was > 0.99 (0.002-0.100 ppm).

Recovery

Recoveries were all > 70% with standard deviations < 20%.

Guidelines and limitations

1. Only parent spirodiclofen was measured. Since hydrolysis during heating for processing into apple sauce might result in considerable amounts of M01 (up to 50%), the study is ~~not~~ appropriate for deriving processing factors for doing risk assessments except for apple sauce.

Conclusion

The method used was validated (recoveries/linearity) for the different fractions and considered acceptable (being > 70% with a relative standard deviation < 20% and correlation coefficient > 0.99, respectively). Residue is associated with peel (>35%). Wet pulp showed CF > 1 and the other fractions showed CF < 1.

B.7.7.2. Overall conclusions on processing

Table B.7.4.2.1 Processing of spirodiclofen after extraction in different apple fractions

Matrix apple	concentration (CF) with heating (up to 100°C, except apple sauce: 155°C, addendum)	concentration (CF) with heating (up to 100°C, DAR)
whole fruit unwashed	0.55 mg/kg	0.028 mg/kg
whole fruit washed	0.65	1.25
wet pomace	3.9	5.9
juice	0.02	<0.71
concentrated juice	0.02	No data
apple sauce	0.02	<0.71
dried fruit	0.02	No data

In the DAR, processing was described during heating (hydrolysis study, parent as well as M01 was measured). From the hydrolysis study it appeared that spirodiclofen can be degraded into M01 at a significant level at pH ~~5-6~~ 4 (up to 50% at ~~100-~~120°C).

The orange data described in this second study were supplementary, since in the DAR only processing for orange marmalade was described.

For apple, the processing factor for wet pomace was similar, while the processing factors for washed fruit, apple sauce and juice were higher in this second study. The higher level spirodiclofen of washed fruits (CF = 1,2-1,3) was only possible by assuming water loss of the fruits.

The ~~higher~~ lower concentration factor in apple sauce and juice ~~was unexpected, since these samples were heated~~ might be due to higher temperatures (155°C for apple sauce compared to 100°C in the study of Nusslein and Huix, evaluated in the DAR) and degradation into M01 was expected to account to a higher extend. However, the studies were not comparable with respect to the initial level of pesticides (0.028 mg and 0.55 mg/kg, respectively). Therefore, the processing factors derived in the second study give more reliable results. The pH which was adjusted in the second study, which might also influence breakdown of spirodiclofen into M01 since degradation is higher at higher pH.

~~Since in all the processing studies with fruits spirodiclofen parent was measured only, and it was assumed that spirodiclofen was not hydrolysed into M01 which is also of toxicological significance, it is assumed to conclude that processing factors which are relevant for risk assessment can not be derived.~~

It is proposed that processing factors can be derived for products processed at temperatures below 100°C, except for apple sauce produced at high temperature (155°C).

B.7.8 Livestock feeding studies

B.7.8.1 feeding studies with cow

The study was evaluated before in the draft assessment report, but is reassessed with regard to the changed residue definition which is now metabolite M01 only, and with regard to the dose levels 3.4x and 10x which were not tabulated in the draft assessment report which should provide more insight at residue levels at 3.4x and 10x dose level.

Ten lactating Holstein dairy cows (one control and three cows per treatment group) were dosed orally with spirodiclofen, once a day after morning milking via a capsule with a balling gun, for 29 consecutive days at a nominal dose rate of 1.38 – 4.14 – 13.8 mg/kg dw feed (3.4x-10x-34x dose) [Krolski, 2001]. The average feed consumption was 30 kg for the control cow and 28 kg for the treated cows and therefore the actual dose rates were 1.29-3.93-13.1 mg/kg dw feed. The animals were 3-5 years old and weighed between 508-782 kg. Milk was collected twice daily during the dosing period. Additionally, a portion of the milk samples from the 10x dose group was separated into cream and whey by centrifugation. On day 29, the animals were slaughtered within 8 hours after the last dose and liver, kidney, muscle and fat were collected. Fat was a composite sample from omental, renal and subcutaneous fat; muscle was a composite sample from loin, round and flank. Samples were stored frozen at ≤ -10 °C for a maximum of 25 days. All tissue and 28-day milk samples from the 34x dose group were analysed by method 109720 (see B.5.2.4 of the draft assessment report). Samples from 3.4x and 10x dose groups were only analysed if residues were found in the higher dose group.

Method verification

Concurrent method recoveries for milk, whey, cream, liver, kidney, muscle and fat are summarized in table B.5.2.4a/b of the draft assessment report. These recoveries are in the required range of 70-120%. Levels in control samples were less than 0.3x the proposed LOQ for each of the analytes (0.004 mg/kg eq in whole milk and whey, 0.01 mg/kg in muscle, fat and cream, 0.05 mg/kg for liver and fat).

Residue analytical method

Questions were raised about the number of recoveries performed for the different matrices of animal origin. Bayer provided a statement to declare that if all data from the original report describing method 109720, it's ILV and the livestock feeding study are taken together, a well documented set of recoveries of each matrix is available (Table B.7.5.1 for spirodiclofen and Table B.7.5.2 for metabolite M01).

Report 109720 describing the original method and report 110477 (ILV) showed the linearity of the detector respons (correlation coefficient > 0.99).

Table B.7.5.1 Recoveries of results for the determination of spirodiclofen using method 109720

matrix	fortification level (mg/kg)	recovery analytical method 109720 (%) (report No. 109720)	recovery ILV Analytical method 109720 (%) (report no. 110477)	recovery cow feeding study (%) (report no. 109898)	Mean ± sdv.
muscle	0.01 0.1	92, 85, 79 103, 82, 74		100, 97	91 ± 8.6 86 ± 15.0
fat	0.01 0.1	80, 95, 87 79, 81, 89		75, 80, 77	82 ± 7.4 83 ± 5.3
kidney	0.05 0.5	92, 78, 71 92, 83, 79		86, 103	86 ± 12.4 85 ± 6.7
liver	0.05 0.1 0.5	115, 112, 72 94, 103, 88	102, 102, 114 *** 111, 113, 98 ***	82, 87	98 ± 16.1 107 ± 8.1 95 ± 7.5
whole milk	0.002 0.004 0.02	108, 103, 88 96, 83, 97 89, 71, 79	 115, 109, 103 109, 107, 101	 103, 107, 97, 94, 98, 96, 108, 113, 98, 102, 106, 107	100 ± 10.4 102 ± 7.8 93 ± 15.6
whey	00.04	-	-	109, 103, 112, 74	100 ± 17.4
cream	0.010	-	-	106, 74, 72, 89	85 ± 15.8

*** The first experiment was not successfully since only the recoveries of spirodiclofen were acceptable. The recoveries of M01 were only around 30%. A new extract and standards were made which were measured on the same day. Only values of this second experiment (spirodiclofen) are depicted in this table.

Table B.7.5.2 Validation results for the determination of M01 using method 109720

matrix	fortification level (mg/kg)	recovery analytical method 109720 (%) (report No. 109720)	recovery ILV Analytical method 109720 (%) (report no. 110477)	recovery cow feeding study (%) (report no. 109898)	Mean ± sdv.
muscle	0.01 0.1	93, 103, 107 91, 105, 118		78, 81	92.4 ± 12.9 105 ± 13.5
fat	0.01 0.1	86, 103, 98 87, 76, 93		79, 92, 85	91 ± 8.9 85 ± 8.6
kidney	0.05 0.5	85, 97, 102 92, 109, 112		108, 103	99 ± 8.7 104 ± 10.8
liver	0.05 0.1 0.5	104, 101, 113 98, 109, 117	103, 101, 102 *** 120, 121, 87 ***	101, 78	100 ± 9.9 109 ± 19.3 108 ± 9.5
whole milk	0.002 0.004 0.02	112, 149, 167 110, 94, 117 86, 75, 76	 105, 107, 102 100, 105, 100	 114, 119, 115, 109, 120, 117, 114, 119, 109, 116, 114, 115	143 ± 28 112 ± 6.8 90 ± 13.1
whey	0.004	-	-	111, 115, 116, 106	112 ± 4.5
cream	0.010	-	-	84, 103, 78, 100	91 ± 12.1

*** The first experiment was not successfully since only the recoveries of spirodiclofen were acceptable. The recoveries of M01 were only around 30%. A new extract and standards were made which were measured on the same day. Only values of this second experiment (M01) are depicted in this table.

Residues

Analysis of milk from the control and 34x dose group at day –1, 0, 4, 8, 12, 16, 20, 24, 26, and 28 days showed that no residues were found in any of the milk samples (<$LOQ_{proposed}$). The results of the 28 day milk samples and the tissues are shown in table B.7.8.3 of the draft assessment report. Parent was not found in whole milk or any of the tissues, except in cream from one cow and fat from two cows at the 34x dose. Metabolite M01 was not found in whole milk or any of the tissues, except in kidney from one cow (3460) at the 34x feeding levels. At the 3x feeding levels, no parent and no metabolite M01 were found in cream, kidney or fat (not shown).

Assessment

Storage stability assessment is not required because samples were analysed within one month. Residue levels found, fall within the provisional measurement ranges for method 109720. The presence of parent compound in animal commodities was not expected from the goat metabolism study. Data on M01 fulfil the new residue definition for animal products which is M01 for monitoring and risk assessment only.

Table B.7.2.1 Residues of M01 in milk and tissues from cows fed with spirodiclofen at a nominal 34x dose. For kidney residue was also measured at a nominal 10x dose and for fat at a 3.4x and 10x dose.

DAT	Commodity	M01, mg eq/kg, 3.4x	M01, mg eq/kg, 10x	M01, mg eq/kg, 34x
28	whole milk	-	-	<0.004[a] (3)
28	whey	-	-	<0.004[a] (3)
28	cream	-	-	<0.01[a] (3)
28	liver	-	-	<0.05[a] (3)
28	kidney	-	<0.05 [a] (3)	<0.05 (2); 0.094; mean 0.059
28	muscle	-	-	<0.01[a] (3)
28	fat	<0.01[a] (3)	<0.01[a] (3)	<0.01[a] (3)

(3) = 3 x the same residue value for each cow

a Each sample was analysed in duplo

- value not given

Conclusion

Only for milk at the lowest spiked level for metabolite M01 (0.002 mg/kg) recovery was outside the range of 70-110% with a relative standard deviation <20%. It was concluded that, taking all the available data from the three different study reports, enough recoveries were determined to assure that the analytical method uses in the lifestock feeding study with cow was well validated.

The residue results from the measurements on M01 may be used for derivation of the MRL, STMR and HR. Since no residues are expected at the anticipated dose level, MRLs will be set at the level of the LOQ.

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs

Pome fruit

MRL for pome was calculated for the data set of Northern Europe (apple only) and for the data set form Southern Europe (apple and paer together). It was questioned by UK whether MRL calculation for the apple data set form Southern Europe and the pear data set of in Southern Europe, as well as for pome fruit from Northern and Southern Europe together, would lead to the same conclusion. Therefore, an additional calculation was performed at separated data sets. Table 7.6.1 (formerly Table B.7.12.2b from the DAR) was completed (*in italic*).

Table B.7.6.1 Derivation for pome of the STMR, HR and MRL as proposed by the rapporteur member state

Commodity	Site	Residues selected (mg/kg)	R I (mg/kg)	R II (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL
apple	N	0.025, 0.035, 0.039, 0.043, 2x0.049, 0.059, 0.077	0.0976	0.1130	0.046	0.077	0.1 mg/kg
pome fruit	N	0.025, 0.035, 0.039, 0.043, 2x0.049, 0.059, 0.077	0.0976	0.1130	0.046	0.077	0.1 mg/kg
apple	S	<0.02, 0.024, 0.046, 0.055	*0.1020*	*0.0965*	*0.035*	*0.055*	*However: : n_{apple}= 4*
pear	S	0.027, 0.035, 0.039, 0.043	*0.0651*	*0.0800*	*0.037*	*0.043*	*However: n_{pear}= 4*
pome fruit	S	<0.02, 0.024, 0.027, 0.035, 0.039, 0.043, 0.046, 0.055	0.0742	0.0905	0.037	0.055	0.1 mg/kg
All data	*N/S*	*< 0.02, 0.024, 0.025, 0.027, 2x0.035, 2x0.039, 2x0.043, 0.046, 2x0.049, 0.055, 0.059, 0.077*	*0.079*	*0.0980*	*0.041*	*0.077*	*0.1 mg/kg*

Animal products

Residues of M01 were found below the LOQ in tissues and milk at a 34 time dose, except for kidney were residues of 0.094 mg/kg were found in only 1 cow. Residues of M01 in kidney at a 3.4x dose were also below LOQ. Samples at 3.4x and 10x dose were not further analysed when no residue above LOQ was found at the near higher dose level.

It is concluded that no residues are expected under practical conditions and MRLs for ruminants can be set at the LOQ:

Animal product	**MRL (mg/kg)**
Milk	0.005
Fat ruminant	0.01
muscle ruminant	0.01
Kidney ruminant	0.05
Liver ruminant	0.05

Since fruit pomace is not fed to pigs and horse, the MRL is proposed for ruminants only.

Conclusion

Data sets from apple and pear (Southern Europe) do not differ significantly (however, consist of only 4 data points each). Residues in the South are slightly lower than in Northern Europe. However, yield the same MRL when calculated and rounded.

MRLs for ruminants can be set based on the 3.4N dose level from the cow feeding study showing residues of M01 < LOQ for all matrices.

B.7.17 References relied on

References in italic were already summarised in the DAR, and are only taken up here since they are mentioned in the text.

Annex point / reference no.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA, 4.2/01	*Mattern, G.C Woodard, D.L.*	*2001*	*Analytical method for the determination of BAJ 2740 and its enol metabolite (BAJ 2510) in animal tissues and milk. Bayer Corporation, Stilwell, KS, USA, report no. 109720, MO-02-017501. Date 14-03-2001 GLP, unpublished*	*Y*	*BCS*
IIA, 4.2/02	*Nelson, S. Hoshowski, J.*	*2001*	*Independent laboratory validation of the "analytical method for the determination of BAJ 2740 and its enol metabolite (BAJ 2510) in animal tissues and milk". Enviro-Test Laboratories, Edmonton, Alberta, Canada, report no. 110477, MO-02-017505. Date 16-07-2001 GLP, unpublished.*	*Y*	*BCS*
IIA, 4.2/03	*Krolski, M.E.*	*2001*	*BAJ 2740 – A 29-day dairy cattle feeding study. [redacted], USA. Report no 109898, MO-01-010656 Date: January 17, 2001 GLP, unpublished*	*Y*	*BCS*
IIA, 4.2/04	Andersch, I.	2005	Statement on validation of analytical method 109720 for determination of BAJ 2740 and its enol metabolite in animal tissues and milk	N	BCS
IIA, 6.1/01	*Babczinski, P.*	*1999a*	*Metabolism of BAJ 2740 in citrus (oranges) after early application Bayer AG, Report No.: MR-226/98 Date: November 2, 1999, Revised: December 13, 2000 GLP, unpublished*	*Y*	*BAY*
6.1/02 6.3/01	Sur, R.	2004	Extraction efficiency testing of the residue method for the determination of BAJ2740 in grapes using aged radioactive residue Report No: MEF-04/453 Bayer Crop Science AG Report	Y	BCS
6.1/03	Zimmer, D Gnielke, A	2004	Addendum 01 to report no. MR-351/99: Validation of the residue analytical method 00568 for the determination of BAJ 2740 in plant materials by LC-MS/MS	Y	BCS
IIA, 6.5/01	*Nüsslein, F. Huix, M.*	*2000e*	*Determination of residues of BAJ 2740 240 SC (as BAJ 2740) on orange in the field in Spain. Processing study (marmalade) Bayer AG, Report No. RA-3020/98; report includes trial no.: 812668 (identical to 1266-98) Date: May 2, 2000 GLP, unpublished*	*Y*	*BAY*
IIA, 6.5/02	*Nüsslein, F. Neigl, A.*	*2000c*	*Determination of residues of BAJ 2740 on apple after spray application of BAJ 2740 240 SC in the field in Germany (fruit, fruit washed, juice, sauce, pomace wet and pomace dried).*	*Y*	*BAY*

Annex point / reference no.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
			Processing study. *Bayer AG, Report No.: RA-3022/98* *report includes study no.:* *812641 (identical to 1264-98)* *Date: February 7, 2000* *GLP, unpublished*		
IIA, 6.5/03	Krolski, M.E	2000	BAJ 2740 240 SC – Magnitude of the residue in orange processed commodities. Report No: 109726	Y	BCS
IIA, 6.5/04	Harbin, A.M	2002	BAJ 2740 240 SC – Magnitude of the residue in apple processed commodities. Report No: 110025	Y	BCS

APPENDIX 1: Exposure model calculations and risk assessment for all applications

1. BASIC APPLICATION INFORMATION

Manual and mechanical upward spraying in pome fruits, stone fruits, citrus and grapes

Product information

Product :	BAJ 2740 SC 240
Purpose:	Acaricide/insecticide
Active substance (a.s.):	spirodiclofen 240 g a.s./l
	Suspension Concentrate (SC)
Package size:	1 L bottle (42.0 mm aperture), 5 L can (54.7 mm aperture)
	and 10 L can (54.7 mm aperture), HDPE

1.1 OPERATOR EXPOSURE

Input data:

Concentration a.s. in formulation	:	240 g a.s. /l
Spray volume	:	1000 - 3000 l/ha
Concentration in spray liquid	:	0.048-0.096 g a.s. /l
Application rate	:	0.096-0.144 kg a.s./ha
		(0.4-0.6 L product/ha)
Treated area manual spraying (15L knapsack)	:	0.15-0.45 ha*

** It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.*

1.2 BYSTANDER EXPOSURE

Input data

Body surface	:	2 m²
Duration	:	1 hour
Ventilation rate	:	1.25 m³/hour
Body weight	:	70 kg

1.3 WORKER EXPOSURE

Exposure calculations for re-entry activities in cultivation of fruits

Input data

Dermal exposure (transfer coefficient)	:	0.45 m^2/hour
Duration of activities	:	6 hour
Body weight	:	70 kg
PPE	:	Gloves with reduction factor 0.1

1.4 RISK ASSESSMENT

Input data

Dermal absorption	:	10 %
Inhalation absorption	:	100 %

2. OPERATOR EXPOSURE

For the present purpose the original UK-POEM and DE (GM) spreadsheets are used. The models differ from each other. The DE (GM) spreadsheet calculates with different reduction factors for PPE while the UK sheet only calculates with gloves as reduction factor.

2.1 External exposure estimates with the UK model

The model is largely based on unpublished studies, carried out in the UK by industry and MAFF. The format of exposure is the volume of spray per unit of time. The exposure during mixing/loading is estimated on the basis of package size, type of formulation and number of operations. The format of exposure for mixing/loading is weight or volume of approximate formulation. For the present purpose, liquids are used in 1 L bottles. 75th percentiles are calculated.

2.1.1 Mechanical upward spraying grapes without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0,4** l/ha	Work rate/day	**15** ha
Application volume	**1000** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0,06	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0,06	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,06	ml/day	121,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	14,4	mg/day	11,6352	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	1,44	mg/day	1,16352	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,0288	mg/day
Percent absorbed	100	%
Absorbed dose	0,0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	2,63232	mg/day
Operator body weight	60	kg
Operator exposure	0,043872	mg/kg bw/day

2.1.2 Mechanical upward spraying in grapes with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	Gloves	PPE during application	Gloves
Dose	**0,4** l/ha	Work rate/day	**15** ha
Application volume	**1000** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0,06	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,006	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,006	ml/day	85,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	1,44	mg/day	8,1792	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,144	mg/day	0,81792	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,0288	mg/day
Percent absorbed	100	%
Absorbed dose	0,0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0,99072	mg/day
Operator body weight	60	kg
Operator exposure	0,016512	mg/kg bw/day

2.1.3 Mechanical upward spraying in pome fruits/stone fruits without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0,6** l/ha	Work rate/day	**15** ha
Application volume	**1500** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0,09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0,09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,09	ml/day	121,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	21,6	mg/day	11,6352	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	2,16	mg/day	1,16352	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,0288	mg/day
Percent absorbed	100	%
Absorbed dose	0,0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	3,35232	mg/day
Operator body weight	60	kg
Operator exposure	0,055872	mg/kg bw/day

2.1.4 Mechanical upward spraying in pome fruits/stone fruits with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**
Formulation type	organic solvent-based		a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %		Dermal absorption from spray	**10** %
Container	1 litre any closure			
PPE during mix/loading	Gloves		PPE during application	Gloves
Dose	**0,6** l/ha		Work rate/day	**15** ha
Application volume	**1500** l/ha		Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0,09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,009	ml/day	85,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	2,16	mg/day	8,1792	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,216	mg/day	0,81792	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,0288	mg/day
Percent absorbed	100	%
Absorbed dose	0,0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1,06272	mg/day
Operator body weight	60	kg
Operator exposure	0,017712	mg/kg bw/day

2.1.5 Mechanical upward spraying in citrus without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0,6** l/ha	Work rate/day	**15** ha
Application volume	**3000** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0,09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0,09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,09	ml/day	121,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,048	mg/ml
Dermal exposure to a.s.	21,6	mg/day	5,8176	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	2,16	mg/day	0,58176	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,048	mg/ml
Inhalation exposure to a.s.	0,0144	mg/day
Percent absorbed	100	%
Absorbed dose	0,0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	2,75616	mg/day
Operator body weight	60	kg
Operator exposure	0,045936	mg/kg bw/day

2.1.6 Mechanical upward spraying in citrus with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**
Formulation type	organic solvent-based		a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %		Dermal absorption from spray	**10** %
Container	1 litre any closure			
PPE during mix/loading	Gloves		PPE during application	Gloves
Dose	**0,6** l/ha		Work rate/day	**15** ha
Application volume	**3000** l/ha		Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0,09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5,2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85,2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,009	ml/day	85,2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,048	mg/ml
Dermal exposure to a.s.	2,16	mg/day	4,0896	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,216	mg/day	0,40896	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,048	mg/ml
Inhalation exposure to a.s.	0,0144	mg/day
Percent absorbed	100	%
Absorbed dose	0,0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0,63936	mg/day
Operator body weight	60	kg
Operator exposure	0,010656	mg/kg bw/day

2.1.7 Manual upward spraying in grapes without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**10**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0,4**	l/ha	Work rate/day	**0,4**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,4	litres product/ha
Work rate	0,4	ha/day
Number of operations	27	/day
Hand contamination	0,27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0,27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2,5	4,5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	64,8	mg/day	9,792	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	6,48	mg/day	0,9792	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,01152	mg/day
Percent absorbed	100	%
Absorbed dose	0,01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	7,47072	mg/day
Operator body weight	60	kg
Operator exposure	0,124512	mg/kg bw/day

2.1.8 Manual upward spraying in grapes with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**
Formulation type	organic solvent-based		a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %		Dermal absorption from spray	**10** %
Container	1 litre any closure			
PPE during mix/loading	Gloves		PPE during application	Gloves
Dose	**0,4** l/ha		Work rate/day	**0,4** ha
Application volume	**1000** l/ha		Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,4	litres product/ha
Work rate	0,4	ha/day
Number of operations	27	/day
Hand contamination	0,27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1,25	2,5	4,5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49,5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,027	ml/day	49,5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	6,48	mg/day	4,752	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,648	mg/day	0,4752	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,01152	mg/day
Percent absorbed	100	%
Absorbed dose	0,01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1,13472	mg/day
Operator body weight	60	kg
Operator exposure	0,018912	mg/kg bw/day

2.1.9 Manual upward spraying in pome fruits, stone fruits, without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0.6** l/ha	Work rate/day	**0.267** ha
Application volume	**1500** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.266666667	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	64.8	mg/day	9.792	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	6.48	mg/day	0.9792	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	7.47072	mg/day
Operator body weight	60	kg
Operator exposure	0.124512	mg/kg bw/day

2.1.10 Manual upward spraying in pome fruits, stone fruits, with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**10**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0,6**	l/ha	Work rate/day	**0,267**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	0,266666667	ha/day
Number of operations	27	/day
Hand contamination	0,27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1,25	2,5	4,5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49,5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,027	ml/day	49,5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,096	mg/ml
Dermal exposure to a.s.	6,48	mg/day	4,752	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,648	mg/day	0,4752	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,096	mg/ml
Inhalation exposure to a.s.	0,01152	mg/day
Percent absorbed	100	%
Absorbed dose	0,01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	1,13472	mg/day
Operator body weight	60	kg
Operator exposure	0,018912	mg/kg bw/day

2.1.11 Manual upward spraying in citrus without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target		
Product	**BAJ 2740 SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**10** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0,6** l/ha	Work rate/day	**0,133** ha
Application volume	**3000** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	0,133333333	ha/day
Number of operations	27	/day
Hand contamination	0,27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0,27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2,5	4,5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,048	mg/ml
Dermal exposure to a.s.	64,8	mg/day	4,896	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	6,48	mg/day	0,4896	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,048	mg/ml
Inhalation exposure to a.s.	0,00576	mg/day
Percent absorbed	100	%
Absorbed dose	0,00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	6,97536	mg/day
Operator body weight	60	kg
Operator exposure	0,116256	mg/kg bw/day

2.1.12 Manual upward spraying in citrus with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**10**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0,6**	l/ha	Work rate/day	**0,133**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0,01	ml
Application dose	0,6	litres product/ha
Work rate	0,133333333	ha/day
Number of operations	27	/day
Hand contamination	0,27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0,027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1,25	2,5	4,5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49,5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0,027	ml/day	49,5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0,048	mg/ml
Dermal exposure to a.s.	6,48	mg/day	2,376	mg/day
Percent absorbed	10	%	10	%
Absorbed dose	0,648	mg/day	0,2376	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0,02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0,048	mg/ml
Inhalation exposure to a.s.	0,00576	mg/day
Percent absorbed	100	%
Absorbed dose	0,00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0,89136	mg/day
Operator body weight	60	kg
Operator exposure	0,014856	mg/kg bw/day

2.2 External exposure estimates with the German model

The German model is based on unpublished studies performed by industry and all carried out in Germany. For mixing/loading the nature of the formulation is an important variable. The format of exposure is mg/kg and the chosen statistic is the geometric mean (GM).

2.2.1 Mechanical upward spraying in grapes

= HIGH_CROP_TRACTOR_MOUNTED =

Treated area per day	**A =**		**8**	**ha/d**	at BBA = 8
Use rate	**R =**		***0,096***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0,0006	0,07	0,008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2,4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0,0006***	0,096	8	0,0004608	mg/pers. x d
Ia = I*a x R x A	0,018	0,096	8	0,013824	mg/pers. x d
		I, in total =		**0,0142848**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2,4***	0,096	8	1,8432	mg/pers. x d
Da/h = D*a/h x R x A	0,7	0,096	8	0,5376	mg/pers. x d
Da/c = D*a/c x R x A	1,2	0,096	8	0,9216	mg/pers. x d
Da/b = D*a/b x R x A	9,6	0,096	8	7,3728	mg/pers. x d
		D, in total =		**10,6752**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0,0004608	-	1	0,0004608	mg/pers. x d
Ia =	0,013824	-	1	0,013824	mg/pers. x d
				0,0142848	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	1,8432	SS 110	0,01	0,018432	mg/pers. x d
Da/h =	0,5376	SS 120	0,01	0,005376	mg/pers. x d
Da/c =	0,9216	SS 420	0,5	0,4608	mg/pers. x d
Da/b =	7,3728	SS 220	0,05	0,36864	mg/pers. x d

					0,853248	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0,0004608	0,0004608	0,0004608	0,0004608
Inhalation: appl.	100%	0,013824	0,013824	0,013824	0,013824
Dermal: m/l	10%	1,8432	0,018432	0,18432	0,0018432
Dermal: appl.	10%	8,832	0,834816	0,8832	0,0834816
			mg/pers./d:	1,0818048	0,0996096
kg bw:	70		mg/kg bw/d:	0,01545435	0,00142299
syst. AOEL:	0,009		**% of AOEL:**	**171,715048**	**15,8110476**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0,08	**Im**
Half mask with comb. filter (m/l)	ST 210	0,02	
Particle filtering half mask (appl.)	ST 120	0,08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0,02	
Protective gloves (m/l)	**SS 110**	**0,01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0,01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0,8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0,5**	
Hood and visor (appl.: high crops)	SS 520	0,05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0,05**	**Da/b**

1.2.2 Manual upward spraying in grapes

For manual spraying with a 15L knapsack, a treated area of 0.15-0.45 ha is used. It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.

= HIGH_CROP_HAND_HELD (HCHH) = **(at BBA)**

Treated area per day	**A =**	***0,45***	**ha/d**	**(1 ha/d)**	
Use rate	**R =**	***0,096***	**kg a.i./ha**		

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	I*a = 0,3	D*a/c = 4,8
I*m	0,05	0,8	0,02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = I*m x R x A	***0,05***	0,096	0,45	0,00216	mg/pers. x d
Ia = I*a x R x A	0,3	0,096	0,45	0,01296	mg/pers. x d
		I, in total =		**0,01512**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0,096	0,45	8,856	mg/pers. x d
Da/h = D*a/h x R x A	10,6	0,096	0,45	0,45792	mg/pers. x d
Da/c = D*a/c x R x A	4,8	0,096	0,45	0,20736	mg/pers. x d
Da/b = D*a/b x R x A	25	0,096	0,45	1,08	mg/pers. x d
		D, in total =		**10,60128**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**			
Im =	0,00216	-	1		0,00216	mg/pers. x d
Ia =	0,01296	-	1		0,01296	mg/pers. x d
					0,01512	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	8,856	SS 110	0,01		0,08856	mg/pers. x d
Da/h =	0,45792	SS 120	0,01		0,0045792	mg/pers. x d
Da/c =	0,20736	SS 420	0,5		0,10368	mg/pers. x d
Da/b =	1,08	SS 220	0,05		0,054	mg/pers. x d
					0,2508192	**mg/pers. x d**

		Estimated exposure		Systemic exposure	
	abs. rate	without PPE	with PPE	without PPE	with PPE
Inhalation: m/l	100%	0,00216	0,00216	0,00216	0,00216
Inhalation: appl.	100%	0,01296	0,01296	0,01296	0,01296
Dermal: m/l	10%	8,856	0,08856	0,8856	0,008856
Dermal: appl.	10%	1,74528	0,1622592	0,174528	0,01622592
			mg/pers./d:	1,075248	0,04020192
kg bw:	70		mg/kg bw/d:	0,01536069	0,00057431
syst. AOEL:	0,009		**% of AOEL:**	**170,674286**	**6,38125714**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0,08	**lm**
Half mask with comb. filter (m/l)	ST 210	0,02	
Particle filtering half mask (appl.)	ST 120	0,08	**la**
Half mask with comb. filter (appl.)	ST 220	0,02	
Protective gloves (m/l)	**SS 110**	**0,01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0,01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0,8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0,5**	
Hood and visor (appl.: high crops)	SS 520	0,05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0,05**	**Da/b**

1.2.3 Mechanical upward spraying in pome fruits, stone fruits and citrus

= HIGH CROP TRACTOR MOUNTED =

Treated area per day	**A =**		**8**	**ha/d**	at BBA = 8
Use rate	**R =**		***0,144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	l*a = 0,018	D*a/c = 1,2
l*m	0,0006	0,07	0,008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2,4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0,0006***	0,144	8	0,0006912	mg/pers. x d
Ia = I*a x R x A	0,018	0,144	8	0,020736	mg/pers. x d
		I, in total =		**0,0214272**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2,4***	0,144	8	2,7648	mg/pers. x d
Da/h = D*a/h x R x A	0,7	0,144	8	0,8064	mg/pers. x d
Da/c = D*a/c x R x A	1,2	0,144	8	1,3824	mg/pers. x d
Da/b = D*a/b x R x A	9,6	0,144	8	11,0592	mg/pers. x d
		D, in total =		**16,0128**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0,0006912	-	1	0,0006912	mg/pers. x d
Ia =	0,020736	-	1	0,020736	mg/pers. x d
				0,0214272	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	2,7648	SS 110	0,01	0,027648	mg/pers. x d
Da/h =	0,8064	SS 120	0,01	0,008064	mg/pers. x d
Da/c =	1,3824	SS 420	0,5	0,6912	mg/pers. x d
Da/b =	11,0592	SS 220	0,05	0,55296	mg/pers. x d
				1,279872	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0,0006912	0,0006912	0,0006912	0,0006912
Inhalation: appl.	100%	0,020736	0,020736	0,020736	0,020736
Dermal: m/l	10%	2,7648	0,027648	0,27648	0,0027648

Dermal: appl.	10%	13,248	1,252224	1,3248	0,1252224
			mg/pers./d:	1,6227072	0,1494144
kg bw:	70		mg/kg bw/d:	0,02318153	0,00213449
syst. AOEL:	0,009		**% of AOEL:**	**257,572571**	**23,7165714**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0,08	**Im**
Half mask with comb. filter (m/l)	ST 210	0,02	
Particle filtering half mask (appl.)	ST 120	0,08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0,02	
Protective gloves (m/l)	**SS 110**	**0,01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0,01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0,8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0,5**	
Hood and visor (appl.: high crops)	SS 520	0,05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0,05**	**Da/b**

1.2.4 Manual upward spraying in pome fruits, stone fruits, and citrus

For manual spraying with a 15L knapsack, a treated area of 0.15-0.45 ha is used. It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.

= HIGH CROP HAND HELD (HCHH) = **(at BBA)**

Treated area per day	**A =**	***0,45***	**ha/d**	**(1 ha/d)**
Use rate	**R =**	***0,144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	I*a = 0,3	D*a/c = 4,8
I*m	0,05	0,8	0,02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = I*m x R x A	***0,05***	0,144	0,45	0,00324	mg/pers. x d
Ia = I*a x R x A	0,3	0,144	0,45	0,01944	mg/pers. x d
		I, in total =		**0,02268**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0,144	0,45	13,284	mg/pers. x d
Da/h = D*a/h x R x A	10,6	0,144	0,45	0,68688	mg/pers. x d
Da/c = D*a/c x R x A	4,8	0,144	0,45	0,31104	mg/pers. x d
Da/b = D*a/b x R x A	25	0,144	0,45	1,62	mg/pers. x d
		D, in total =		**15,90192**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0,00324	-	1	0,00324	mg/pers. x d
Ia =	0,01944	-	1	0,01944	mg/pers. x d
				0,02268	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	13,284	SS 110	0,01	0,13284	mg/pers. x d
Da/h =	0,68688	SS 120	0,01	0,0068688	mg/pers. x d
Da/c =	0,31104	SS 420	0,5	0,15552	mg/pers. x d
Da/b =	1,62	SS 220	0,05	0,081	mg/pers. x d
				0,3762288	**mg/pers. x d**

		Estimated exposure		Systemic exposure	
	abs. rate	without PPE	with PPE	without PPE	with PPE
Inhalation: m/l	100%	0,00324	0,00324	0,00324	0,00324
Inhalation: appl.	100%	0,01944	0,01944	0,01944	0,01944
Dermal: m/l	10%	13,284	0,13284	1,3284	0,013284
Dermal: appl.	10%	2,61792	0,2433888	0,261792	0,02433888
			mg/pers./d:	1,612872	0,06030288
kg bw:	70		mg/kg bw/d:	0,02304103	0,00086147
syst. AOEL:	0,009		% of AOEL:	256,011429	9,57188571

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0,08	**lm**
Half mask with comb. filter (m/l)	ST 210	0,02	
Particle filtering half mask (appl.)	ST 120	0,08	**la**
Half mask with comb. filter (appl.)	ST 220	0,02	
Protective gloves (m/l)	**SS 110**	**0,01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0,01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0,8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0,5**	
Hood and visor (appl.: high crops)	SS 520	0,05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0,05**	**Da/b**

2.3 Risk assessment for operator exposure

Risk assessment was performed using the 75th percentile from the UK-model (UK-75th) and the geometric mean from the German model (DE-GM). The external exposure values were corrected for percentage absorption (see 1.4 of this appendix) to obtain internal exposure values.

Appendix table 2.3.1 **Operator internal exposure and risk assessment**

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying in grapes						
UK- 75th	Respiratory	0.029	0.029	0.54	5	5
	Dermal	2.60	0.96	0.54	481	178
	Total exposure	**2.63**	**0.99**	**0.54**	**487**	**183**
DE- GM	Respiratory	0.01	0.01	0.63	2	2
	Dermal	1.07	0.09	0.63	169	14
	Total exposure	**1.08**	**0.10**	**0.63**	**172**	**16**
Mechanical upward spraying in pome fruits, and stone fruits						
UK- 75th	Respiratory	0.03	0.03	0.54	5	5
	Dermal	3.32	1.03	0.54	615	191
	Total exposure	**3.35**	**1.06**	**0.54**	**621**	**197**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	1.60	0.13	0.63	254	20
	Total exposure	**1.62**	**0.15**	**0.63**	**258**	**24**
Mechanical upward spraying in citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	3	3
	Dermal	2.74	0.62	0.54	508	116
	Total exposure	**2.76**	**0.64**	**0.54**	**510**	**118**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	1.60	0.13	0.63	254	20
	Total exposure	**1.62**	**0.15**	**0.63**	**258**	**24**
Manual upward spraying in grapes						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL-systemic (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
	Dermal	7.46	1.12	0.54	1381	208
	Total exposure	**7.47**	**1.13**	**0.54**	**1383**	**210**
DE- GM	Respiratory	0.02	0.02	0.63	2	2
	Dermal	1.06	0.03	0.63	168	4
	Total exposure	**1.08**	**0.04**	**0.63**	**171**	**6**
Manual upward spraying in pome fruits, and stone fruits						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2
	Dermal	7.46	1.12	0.54	1381	208
	Total exposure	**7.47**	**1.13**	**0.54**	**1383**	**210**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	1.59	0.04	0.63	252	6
	Total exposure	**1.61**	**0.06**	**0.63**	**256**	**10**
Manual upward spraying in citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	1	1
	Dermal	6.97	0.89	0.54	1291	164
	Total exposure	**6.98**	**0.89**	**0.54**	**1292**	**165**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	1.59	0.04	0.63	252	6
	Total exposure	**1.61**	**0.06**	**0.63**	**256**	**10**

3. Bystander exposure

3.1 External exposure estimates

Manual and mechanical upward spraying

For bystander exposure during manual or mechanical upward spraying, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model will be used. The input data are presented in paragraph 1.2 of this appendix.

Dermal exposure: a maximum of 15% of the application rate (kg/ha) on an assumed body surface of 2 m^2

Inhalation exposure: 0.06 ml spraying liquid per m^3. For inhalation exposure, duration of 1 hour and ventilation rate of 1.25 m^3/hour will be assumed. Thus, bystander exposure is estimated as follows.

Dermal exposure = 0.15 x 0.144 kg/ha x 2 m^2 x 100 (correction for kg/ha to mg/m^2)
= 4.3 mg a.s./day (61 µg/kg bw/day).

Inhalation exposure = 0.06 ml/m^3 x 1 hour x 1.25 m^3/hour x 0.096 mg/ml
= < 0.01 mg a.s./day (< 0.14 µg/kg bw/day).

In the GAP information, it is stated that BAJ 2740 SC 240 should be applied only once during the grow season. Thus, bystander exposure will be limited to a maximum of a few days per year.

3.2 Risk assessment for bystanders

Risk assessment was performed using the 90 th percentile from EUROPOEM II. The external exposure values were corrected for percentage absorption (see 1.4 of this appendix) to obtain internal exposure values.

Appendix table 3.2.1 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic (mg a.s./day)	%AOEL
Exposure during mechanical upward spraying on pome/stone fruit, citrus, and grapes			
Respiratory	<0.01	0.63	<1
Dermal	0.43	0.63	68
Total exposure	**0.44**	**0.63**	**70**

4. Worker exposure

4.1 External worker exposure estimations

For the harvesting of fruits from tree crops the dermal exposure level can be estimated in an indirect way by the estimated dislodgeable foliar residue and a task-specific transfer factor for the re-entry activities using the following equations:

DFR_0 = (AR / LAI) x 100 (I)

Where

DFR $_0$ = initial foliar residue (mg/m^2)

AR = application rate (kg/ha)

LAI = leaf area index (m^2 foliar surface / m^2 ground surface)

Using a maximum application rate of 0.144 mg as/ha and an leaf area index of 2 m^2/ m^2 as a worst case assumption, the initial DFR is calculated to be:

DFR $_0$ (mg/m^2) = (AR (kg as/ha) / LAI (m^2/ m^2)) x 100

= (0.144 / 2) x 100

= 7.2 mg as/ m^2

A pre-harvest interval of 14 days is given. However, as a worst-case estimate a situation with no dissipation will be considered because data on dissipation is not present.

DE = DFR x TC x Df x T (II)

In which

DE = dermal exposure (mg a.s./day)

DFR = dislodgeable foliar residue at time of re-entry (mg/m^2)

TC = task specific transfer factor (m^2/hour)

Df = Dissipation function (no units, 1 when no dissipation is assumed)

T = duration of the activity considered (hour/day)

A typical transfer coefficient (TC) of 0.45 m^2/h, as proposed for EUROPOEM II, and a workday of 6 hours are presumed. This way, the dermal exposure is estimated to be:

DE (mg a.s./day) = DFR (mg/m^2) x TC (m^2/hour) x Df (no units) x T (hour/day)

= 7.2 x 0.45 x 1 x 6

= 19 mg as/day

The inhalation exposure is not quantifiable with this method.

Discussion of the results

The estimated exposures must be considered relevant for the crops with the highest levels of contact with the crop and thus highest levels of exposure. The results for worker exposure are considered to reflect a worst case situation. Exposure levels will be lower for crops with only minor contact between crop and worker during re-entry activities or with increased PHI. The exposure levels are upper bound for another reason; dissipation is considered not to occur

between application and crop activities.

In the GAP information, it is stated that BAJ 2740 SC 240 should be applied only once during the grow season. Thus, worker exposure will probably be limited to a short period of re-entry tasks shortly after application.

4.2 Risk assessment for worker exposure

The external exposure values were corrected for percentage absorption (see 1.4 of this appendix) to obtain internal exposure values.

Appendix table 4.2.1 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic (mg a.s./day)	% AOEL	
	without PPE	with PPE		without PPE	with PPE
Exposure after mechanical upward spraying on apples and pears					
Respiratory	-	-	-	-	-
Dermal	1.9	0.19	0.63	302	30
Total exposure	1.9	0.19	0.63	302	30

- The inhalation exposure is not quantifiable with this method.

European Commission

SPIRODICLOFEN

ADDENDUM B8 B9

EPCO-MEETINGS 26-27

Rapporteur Member State: The Netherlands

May 2005, revised September 2006

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

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LEVEL 2

Spirodiclofen

OVERALL CONCLUSIONS

2.5 Fate and behaviour in the environment

2.5.1 Definition of the residues relevant to the environment

The metabolites BAJ 2740-enol, BAJ 2740-ketohydroxy, BAJ 2740-dihydroxy and 2,4-dichlorobenzoic acid were formed at >10% AR under aerobic laboratory conditions in soil treated with Spirodiclofen. The metabolite BAJ 2740-enol was formed at >10% AR in the water phase of water/sediment systems treated with Spirodiclofen.

The residue definitions for monitoring are therefore as follows:

For risk assessment

Soil: spirodiclofen, BAJ 2740-enol, BAJ 2740-ketohydroxy, BAJ 2740-dihydroxy, 2,4-dichlorobenzoic acid

Groundwater: spirodiclofen, BAJ 2740-enol, BAJ 2740-ketohydroxy, BAJ 2740-dihydroxy, 2,4-dichlorobenzoic acid

Surfacewater and sediment: Spirodiclofen, BAJ 2740-enol

Air: Spirodiclofen

For monitoring

Soil, groundwater, surfacewater and sediment, air
Spirodiclofen

2.5.2 Fate and behaviour in soil

PECsoil

Authorisation is requested for treatment of orchards and vines involving a single seasonal application at 0.144 and 0.096 kg a.s./ha, respectively. Initial PEC_S values were calculated under the following assumptions: 50% crop interception; homogeneous distribution in the top 5 cm layer; a soil bulk density of 1.5 g/cm^3. Initial PEC_S of the metabolites was derived from that of the a.s., with corrections for the difference in molecular mass and the maximum percentage at which the metabolite was formed from the parent substance during laboratory studies. Time weighted average (TWA) PECs values were based on worst case DT50 (lab) values.

Table A2.5.2-1 Initial and TWA PECs (mg/kg) of Spirodiclofen and major metabolites.

day after appln.	Spirodiclofen		BAJ 2740-enol		BAJ 2740-ketohydroxy		BAJ 2740-dihydroxy		2,4-dichloro-benzoic acid	
	actual	TWA	actual	TWA	actual	TWA	actual	TWA	actual	TWA
orchards (0.144 kg a.s./ha)										
0	0.096	-	0.038	-	0.034	-	0.013	-	0.018	-
1	0.091	0.093	0.035	0.037	0.032	0.033	0.012	0.013	0.017	0.017
2	0.086	0.091	0.033	0.035	0.031	0.032	0.012	0.012	0.016	0.017
4	0.078	0.086	0.029	0.033	0.028	0.031	0.012	0.012	0.014	0.016
7	0.066	0.080	0.023	0.030	0.024	0.029	0.011	0.012	0.011	0.014
21	0.031	0.058	0.009	0.020	0.012	0.021	0.008	0.010	0.005	0.010
28	0.022	0.050	0.005	0.017	0.009	0.018	0.007	0.009	0.003	0.008
50	0.007	0.034	0.001	0.010	0.003	0.013	0.004	0.007	0.001	0.005
100	0.0005	0.018	0.000	0.005	0.000	0.007	0.001	0.005	0.000	0.003
grapevine (0.096 kg a.s./ha)										
0	0.064	-	0.025	-	0.023	-	0.009	-	0.012	-
1	0.061	0.062	0.024	0.024	0.022	0.022	0.008	0.008	0.011	0.011
2	0.058	0.061	0.022	0.024	0.022	0.022	0.008	0.008	0.010	0.011
4	0.052	0.058	0.019	0.022	0.021	0.022	0.008	0.008	0.009	0.010
7	0.044	0.053	0.015	0.020	0.019	0.021	0.007	0.008	0.008	0.010
21	0.021	0.039	0.006	0.013	0.013	0.018	0.005	0.007	0.003	0.007
28	0.014	0.033	0.003	0.011	0.011	0.016	0.004	0.006	0.002	0.006
50	0.004	0.022	0.001	0.007	0.006	0.013	0.003	0.005	0.001	0.004
100	0.0003	0.012	0.000	0.004	0.002	0.008	0.001	0.003	0.000	0.002

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

Routes of exposure.

For applications in orchards and grapevine, the routes of exposure are considered to be:

- consumption of oversprayed insects (birds) or short grass (mammals) with residues of Spirodiclofen (short- and long-term);
- consumption of surface water containing residues of Spirodiclofen (short-term);
- consumption of fish contaminated with residues of Spirodiclofen (long-term);
- consumption of earthworms contaminated with residues of Spirodiclofen (long-term).

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC (Working Document Sanco/4145/2000, September 2002).

2.6.1.1 Birds

Avian toxicity data.

The acute oral LD50 of Spirodiclofen and the metabolites BAJ 2740-enol and BAJ 2740-4-hydroxy-enol for bobwhite quail was >2000 mg a.s./kg body weight.

For bobwhite quail and mallard (ducklings), the dietary LC50 of Spirodiclofen was >5000 mg a.s./kg diet, equivalent to >1061 and >2274 mg a.s./kg bw/day, respectively.

Two reproductive toxicity studies with Spirodiclofen (bobwhite quail and mallard duck) were submitted. In the study with bobwhite quail there were no effects on reproductive parameters up to and including the highest test level of 720 mg a.s./kg diet, equivalent to 51 mg a.s./kg bw/day. In the study with mallard duck the NOEC was 734 mg as/kg diet, equivalent to 111 mg as/kg bw/d.

Risk assessment (active substance).

The risk assessment is based on a bird of body weight (BW) 10 g feeding on small insects, with a daily food intake (DFI) of 10.4 g fresh material/day, and a daily water intake (DWI) of 2.7 mL/day. The PECsw are the initial PECsw values. The PEC_{FEED} for acute intake of insects represents 90th percentile values in agreement with the guidance in Sanco/4145/2000. For the short-term risk and chronic assessment the arithmetic mean residue values are taken for residues in insects, as

it is assumed that in the course of some days birds will not always be exposed to insects contaminated with Spirodiclofen but also to uncontaminated food. The ETEs are calculated as PEC_{FEED}*DFI/BW or as PECsw*DWI/BW.

The risk of bio-accumulation was considered for the routes earthworm and fish. The assessment was based on a bird weighing 100 g feeding exclusively on earthworms, with a DFI of 113 g fresh material/day, or on a fish-eating bird weighing 1000 g with a DFI of 206 g fish/day. The BCF for earthworms was calculated as BCF = (0.84+0.01*Pow)/(f_{oc}*Koc), where Koc = 31037 L/kg and f_{oc} = 0.02 (standard value). Pow, calculated from logPow = 5.83, was 676083. The resulting BCF of Spirodiclofen for earthworms was 10.9 kg earthworm fresh weight per kg soil dry weight. Residue levels in earthworms were calculated from the 21-day TWA PECsoil as follows: PECworm = PECsoil*BCFworm.
The BCF of Spirodiclofen for fish is the experimentally determined value of 491 L/kg wet weight/day (based on total radioactivity). Residue levels in fish were calculated from the worst case 21-day TWA PECsw as follows: PECfish = PECsw*BCFfish.

Residue values in food and water, ETE and TER values are presented in Table 2.6.1.1-1.

Table 2.6.1.1-1. Toxicity Exposure Ratios for exposure of birds to Spirodiclofen due to consumption of contaminated insects and drinking water

appln.	Time scale	Toxicity endpoint (mg a.s./kg bw/day)	route	PEC_{FEED} or PEC_{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TER
orchard	acute	LD50: >2000	insects	7.5	7.8	>256
	acute	LD50: >2000	water	0.014	0.0038	>529120
vine	acute	LD50: >2000	insects	5.0	5.2	>385
	acute	LD50: >2000	water	0.0026	0.00069	>2889706
orchard	short-term	LC50: >1061	insects	4.2	4.3	>247
vine	short-term	LC50: >1061	insects	2.8	2.9	>366
orchard	long-term	NOEC: 51	insects	4.2	4.3	12
vine	long-term	NOEC: 51	insects	2.8	2.9	18
orchard	long-term	NOEC: 51	earthworms	0.63	0.71	72
	long-term	NOEC: 51	fish	0.756	0.16	319
vine	long-term	NOEC: 51	earthworms	0.42	0.47	108
	long-term	NOEC: 51	fish	0.15	0.03	1700

TER values in Table 2.6.1.1-1 are all above the Annex VI 91/414 EEC trigger of 10 for acute and short-term exposure and 5 for long-term exposure. Hence, the risk to birds is acceptable.

Risk assessment (metabolite BAJ 2740-enol).

BAJ 2740-enol was a major metabolite in soil and water. BAJ 2740-enol was also found in rat and goat orally dosed with parent Spirodiclofen, it was a major metabolite in fish exposed to Spirodiclofen, and it was found in plants treated with Spirodiclofen. The primary step in the metabolism of Spirodiclofen was apparently always ester cleavage yielding BAJ 2740-enol. Although no metabolism study with parent Spirodiclofen in laying hens was submitted, it is reasonable to assume, that BAJ 2740-enol is also formed in birds dosed with parent Spirodiclofen. Hence the toxicity of this metabolite to birds is considered to have been covered by the studies with the parent compound.

The risk of exposure of birds to BAJ 2740-enol in oversprayed insects and contaminated surface water is considered to be low since the risk for the parent was low and PEC values for BAJ 2740-enol are lower than for the parent. The long-term risk assessment for bio-accumulation due to intake of contaminated earthworms and fish is addressed below (worst case calculation).

Based on the mean Koc value of 17.8 L/kg and logPow = 3, the BCF of BAJ 2740-enol for earthworms was estimated to be 30 kg earthworm fresh weight per kg soil dry weight. Based on log Pow = 3, the BCF of BAJ 2740-enol for fish is estimated to be 71 L/kg wet weight, according to the formula log BCF=0.85*logPow-0.7 (USES 2.0). Residue levels in earthworms were calculated from the worst case 21-day TWA PECsoil (0.020 mg/kg), and in fish from worst case PECsw value (9.0 µg/L). The NOEC used for risk assessment is that of the parent (51 mg/kg bw/day). Table 2.6.1.1-2 summarises the results.

Table 2.6.1.1-2. Long-term Toxicity Exposure Ratios (worst case) for exposure of birds to BAJ 2740-enol due to consumption of contaminated earthworms and fish

appln.	Time scale	Toxicity endpoint (mg a.s./kg bw/day)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
orchard	long-term	NOEC: 51	earthworms fish	0.60 0.64	0.68 0.13	75 389

TERlt values are all above the Annex VI trigger of 5. Hence, the risk to birds is acceptable.

Risk assessment (metabolites 2,4-dichlorobenzoic acid, BAJ 2740-ketohydroy and BAJ 2740-dihydroxy).

2,4-Dichlorobenzoic acid was a major soil metabolite. It was also found in rats orally dosed with parent Spirodiclofen, but not in goat milk and tissues, not in fish and also not in plants treated with Spirodiclofen. It is considered uncertain therefore, that this metabolite is formed in birds dosed with parent Spirodiclofen, and that its toxicity to birds has been covered by the studies with the parent compound. BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy were major soil metabolites, but they were not found in rats, goat milk and tissues, fish and plants treated with Spirodiclofen. There is no evidence that the toxicity of these metabolite to birds has been covered by the studies with the parent compound.

Birds can be exposed to these 3 soil metabolites by consumption of contaminated earthworms. The mean Koc value of BAJ 2740-ketohydroxy, BAJ 2740-dihydroxy and 2,4-dichlorobenzoic acid is 612, 51.3 and 7.3 L/kg respectively. Experimentally determined maximum logPow values for these metabolites are 4.7 (unbuffered water), 3.67 (pH 9) and $\leq$1.74 (unbuffered water), respectively. Based on logPow $\leq$1.74, i.e. <3, the long-term risk for bio-accumulation of 2,4-dichlorobenzoic acid due to intake of contaminated earthworms is considered to be acceptable. Using the values of Koc and logPow mentioned, the BCF of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy for earthworms was estimated to be 41 and 46 kg earthworm fresh weight per kg soil dry weight respectively. Residue levels in earthworms were calculated from the 21-day TWA PECsoil.

Residue values of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy in earthworms and ETE values are presented in Table 2.6.1.1-3, together with an estimate of the TER based on the NOEC of the parent (51 mg/kg bw/day), and the minimum NOEC value which would give TER >5.

Table 2.6.1.1-3 PEC_{FEED} and ETE values for exposure of birds to BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy due to consumption of contaminated earthworms

compound	appln.	dose (kg as/ha)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TER	TER >5 if NOEC > (mg/kg bw/d)
BAJ 2740-ketohydroxy	orchard vine	0.144 0.096	earthworms earthworms	0.86 0.57	1.0 0.64	51 80	5.0 3.2
BAJ 2740-dihydroxy	orchard vine	0.144 0.096	earthworms earthworms	0.41 0.32	0.46 0.36	111 142	2.3 1.8

Based on the NOEC of the parent, TERlt values of the metabolites are all far above the Annex VI 91/414 EEC trigger of 5. Risk indicators (TERlt) for the parent were clearly on the safe side for birds (TERlt =72). The toxicity of metabolites is usually less than that of the parent. Toxicity tests with the metabolites under consideration were only performed on earthworms, microflora (nitrification test), Collembola and (BAJ 2740-ketohydroxy) on rat. No toxicity was observed in these organisms. The primary metabolite BAJ 2740-enol however was more extensively tested. As for the parent, BAJ 2740-enol was not toxic to birds (LD50 >2000 mg/kg bw). It was about 3 orders of magnitude less toxic than the parent to fish, Daphnia and algae, and 2 orders of magnitude less toxic than the parent to Chironomus. It is likely that the low toxicity of BAJ 2740-enol for aquatic organisms compared to that of the parent is due to the loss of a structural toxophoric unit, which is also absent in the two metabolites under consideration. It can be derived from the data in the above table, that the TERlt of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy would only be <5 in case the NOEC to birds of the metabolite would be lower than that of the parent (51 mg/kg bw/day) by a factor of at least 10 and 22, respectively.

An increased toxicity of the metabolites relative to the parent is considered unlikely, based on the foregoing. Hence, the long-term risk to birds as a result of bio-accumulation of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy due to intake of contaminated earthworms is considered to be acceptable.

2.6.1.2 Mammals

Risk assessment (active substance).

The risk assessment is based on a herbivorous mammal of body weight (BW) 25 g feeding exclusively on short grass, with a daily food intake (DFI) of 34.8 g fresh material/day, and a daily water intake (DWI) of 3.6 mL/day. The ETEs are calculated as PEC_{FEED}*DFI/BW or as PECsw*DWI/BW. The PECsw are the initial PECsw values. The PEC_{FEED} for acute intake is corrected for a crop interception factor of 0.4 and represents 90th percentile values. For the short-term risk and chronic assessment the arithmetic mean residue values are taken for residues in short grass, as it is assumed that in the course of some days mammals will not always be exposed to short grass contaminated with Spirodiclofen but also to uncontaminated food. The long-term residue values are time-weighted over a period of 3 weeks assuming a DT50 of 10 days, which leads to a TWA correction factor of the initial residue values of 0.53.

The risk of bio-accumulation was considered for the routes earthworm and fish. The assessment was based on a mammal weighing 10 g feeding exclusively on earthworms, with a DFI of 14 g fresh material/day, or on a fish-eating mammal weighing 3000 g with a DFI of 390 g fish/day. The acute LD50 is >2500 mg a.s./kg bw. The toxicity value used for the First Tier long-term risk assessment is taken from the 2-generation study in rat (overall NOEC 70 mg a.s./kg diet, equivalent to 6.0 mg as/kg bw/d).

Residue values in food and water, ETE and TER values are presented in Table 2.6.1.2-1.

Table 2.6.1.2-1. Toxicity Exposure Ratios (First Tier) for exposure of mammals to Spirodiclofen due to consumption of contaminated short grass and drinking water.

appln.	Time scale	Toxicity endpoint (mg a.s./kg bw/day)	route	PEC_{FEED} or PEC_{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TER
orchard	acute	LD50: >2500	short grass	12.2	17.0	>147
	acute	LD50: >2500	water	0.014	0.0020	1245876
vine	acute	LD50: >2500	short grass	8.1	11.3	>221
	acute	LD50: >2500	water	0.0026	0.00037	6804159
orchard	long-term	NOEC: 6.0	short grass	3.5	4.9	**1.2**
vine	long-term	NOEC: 6.0	short grass	2.3	3.3	**1.8**
orchard	long-term	NOEC: 6.0	earthworms	0.63	0.88	6.8
	long-term	NOEC: 6.0	fish	0.756	0.10	60
vine	long-term	NOEC: 6.0	earthworms	0.42	0.59	10.2
	long-term	NOEC: 6.0	fish	0.15	0.02	300

TER values in Table 2.6.1.2-1 are above the Annex VI trigger of 10 for acute and short-term exposure and 5 for long-term exposure to contaminated earthworms and fish. Hence, the risk to mammals via these routes is acceptable. TERlt values for exposure to short grass are below the Annex VI trigger of 5, however. Hence, the risk assessment needs to be refined.

No DT50 values for decline of residues in treated grass were determined during field trials, but DT50 values in treated apple and grapevine foliage were 7 and 14 days, respectively. The mean value of 10.5 days is not essentially different from the value of 10 days used above. Due to dilution by growth, however, residues are likely to dissipate faster from the faster growing grass than from fully developed apple and vine foliage.

The NOEC used in the First Tier assessment (70 mg a.s./kg diet, 6.0 mg a.s./kg bw/day) was based on statistical significance of effects at the LOEC (350 mg a.s./kg diet) on body weight of F0 male adults (5.2% during week 12-16) and pup weight (3.2% at birth and 5.6% during lactation), and on histopathological findings in F0 and F1 adults (slight increase in vacuolisation of the adrenals). In a developmental toxicity study in rat however, embryo/foetal development (including foetal weight) was unaffected after 14 daily dose administrations to the pregnant female up to the highest test dose of 1000 mg/kg bw/day. This indicates that the effect on initial pup weight, recorded in the 2-generation study at 350 mg a.s./kg diet, was due to the prolonged exposure of the parent animals to Spirodiclofen. Under a practical scenario involving a single seasonal treatment only, continuous exposure to constant levels of Spirodiclofen for 16 weeks is unlikely to occur due to decline of residues. Besides the slight reduction of pup weight, the only other effect observed in F1 animals fed 350 mg a.s./kg diet was a slight increase in vacuolisation of the adrenals of adult rats.

As there were no other effects at the dose level of 350 mg a.s./kg diet, it is considered acceptable to set the ecotoxicologically relevant NOEC for refined risk assessment at 350 mg a.s./kg diet,

corresponding with 29.6 mg a.s./kg bw/day. Residue values in food, ETE and TERlt values for the refined assessment are presented in Table 2.6.1.2-2.

Table 2.6.1.2-2. Long-term Toxicity Exposure Ratios (Refined Assessment) for exposure of mammals to Spirodiclofen due to consumption of contaminated short grass

appln.	Time scale	Toxicity endpoint (mg a.s./kg bw/day)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
orchard vine	long-term long-term	NOEC: 29.6 NOEC: 29.6	short grass short grass	3.5 2.3	4.9 3.3	6.0 9.0

TERlt values in Table 2.6.1.2-2 are all above the Annex VI trigger of 5. Hence, the long-term risk to mammals is considered to be acceptable.

Risk assessment (metabolite BAJ 2740-enol).

BAJ 2740-enol was a major metabolite in soil and water, and in rats orally dosed with parent Spirodiclofen. Hence the toxicity of this metabolite to mammals is considered to have been covered by the studies with the parent compound.

The risk of exposure of mammals to BAJ 2740-enol in oversprayed short grass and contaminated surface water is considered to be low since the risk for the parent was low and PEC values for BAJ 2740-enol are lower than for the parent. The long-term risk assessment for bio-accumulation is addressed separately below for the routes earthworms and fish (worst case calculations for early application in orchards).

The NOEC used for risk assessment is that of the parent (First Tier value of 6.0 mg/kg bw/day). Residue levels in earthworms and fish and ETE and TERlt values are presented in Table 2.6.1.2-3.

Table 2.6.1.2-3. Long-term Toxicity Exposure Ratios (worst case) for exposure of mammals to BAJ 2740-enol due to consumption of contaminated earthworms and fish

appln.	Time scale	Toxicity endpoint (mg a.s./kg bw/day)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
orchard	long-term	NOEC: 6.0	earthworms fish	0.60 0.64	0.84 0.08	7.1 75

TERlt values are all above the Annex VI trigger of 5. Hence, the risk to mammals is acceptable.

Risk assessment (metabolites 2,4-dichlorobenzoic acid, BAJ 2740-ketohydroy and BAJ 2740-dihydroxy).

2,4-Dichlorobenzoic acid was a major soil metabolite. It was also found in the excreta of rats orally dosed with parent Spirodiclofen, but only in low amounts (<2% AR). Hence exposure of mammals treated with Spirodiclofen to this metabolite was too low to be certain that its toxicity was addressed during studies with parent substance. Based on logPow $\leq$ 1.74, i.e. <3, the long-

term risk for bio-accumulation of 2,4-dichlorobenzoic acid due to intake of contaminated earthworms is considered to be acceptable.

BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy were major soil metabolites, but they were not found in rats, treated with Spirodiclofen. There is no evidence that the toxicity of these metabolite to mammals has been covered by the studies with the parent compound. Mammals can be exposed to these 2 soil metabolites by consumption of contaminated earthworms. BCF values of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy and earthworms were estimated to be 41 and 46 kg earthworm fresh weight per kg soil dry weight respectively.

Residue levels in earthworms, calculated from the 21-day TWA PECsoil and ETE values are presented in Table 2.6.1.2-4, together with an estimate of the TER based on the NOEC of the parent (29.6 mg/kg bw/day) and the minimum NOEC value which would give TER >5.

Table 2.6.1.2-4 PEC_{FEED} and ETE values for exposure of mammals to BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy due to consumption of contaminated earthworms

compound	appln.	dose (kg as/ha)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TER	TER >5 if NOEC > (mg/kg bw/d)
BAJ 2740-ketohydroxy	orchard	0.144	earthworms	0.86	1.2	25	5.9
	vine	0.096	earthworms	0.57	0.80	37	4.0
BAJ 2740-dihydroxy	orchard	0.144	earthworms	0.41	0.57	52	2.9
	vine	0.096	earthworms	0.32	0.45	66	2.2

Based on the NOEC of the parent, TERlt values of the metabolites are all above the Annex VI 91/414 EEC trigger of 5. Risk indicators (TERlt) for the parent were safe for mammals (TERlt =8.7). The toxicity of metabolites is usually less than that of the parent. Toxicity tests with the metabolites under consideration were only performed on earthworms, microflora (nitrification test), Collembola and (BAJ 2740-ketohydroxy) on rat. No toxicity was observed in these organisms. The primary metabolite BAJ 2740-enol however was more extensively tested. As for the parent, BAJ 2740-enol was not toxic to birds (LD50 >2000 mg/kg bw). It was about 3 orders of magnitude less toxic than the parent to fish, Daphnia and algae, and 2 orders of magnitude less toxic than the parent to Chironomus. It is likely that the low toxicity of BAJ 2740-enol for aquatic organisms compared to that of the parent is due to the loss of a structural toxophoric unit, which is also absent in the two metabolites under consideration. It can be derived from the data in the above table, that the TERlt
of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy would only be <5 in case the NOEC to mammals of the metabolite would be lower than that of the parent (29.6 mg/kg bw/day) by a factor of at least 5 and 10, respectively. An increased toxicity of the metabolites relative to the parent is considered unlikely, based on the foregoing. Hence, the long-term risk to mammals as a result of bio-accumulation of BAJ 2740-ketohydroxy and BAJ 2740-dihydroxy due to intake of contaminated earthworms is considered to be acceptable.

Possible endocrine effects

There was a comment from the UK (reporting table 5(19)): *Given the concerns expressed in the*

mammalian toxicology section regarding the mechanisms of toxicity and possible endocrine effects of the a.s. and the -enol metabolite, we would liked to have seen some discussion here as to the suitability of the avian reproduction test and resulting end-point to address all the potential for reproductive effects in birds. It may well be a suitable test and end-point but some clarification would be welcome.

Clarification:
In the mammalian toxicology section it was concluded that spirodiclofen has been shown to disturb the endocrine balance by interfering with steroid hormone synthesis, not by a direct specific effect, but at the level of general biochemical pathways (Krebs cycle and pyruvate/citrate shuttle).

Since the effect is non-specific, the nature of any effects caused is not well predictable.In teratogenicity studies in rabbit and rat, up to the highest tested dose of 1000 mg/kg bw/day no effects were observed which are specifically associated with disturbance of the endocrine balance. In the 2-generation reproduction study in rat, sperm count in F0 male rats was unaffected at all dosages, but spermatogenesis was reduced by 18-23% in F1 male rats at the highest test dose of 134.8 mg/kg bw/day (NOAEL for reproduction based on this effect 26.2 mg/kg bw/day). This reduced spermatogenesis however did not lead to any effects on litter size, survival index or sex ratio in the F2 pups. The NOAEL for systemic effects in this study was <5.2 mg/kg bw/day based, amongst other things, on decreased body weights of parental male F0 rats.

The findings from the 2-generation reproduction study in rat suggest that endocrine effects (not leading to effects on reproductive success) occur at a much higher dose than systemic effects. The reproduction study in bobwhite quail showed an unequivocal lack of toxicity of spirodiclofen to male and female parental birds (mortality, behaviour, feed consumption, body weight, gross pathology), up to the highest tested dose of 51 mg a.s./kg bw/day.

The one-generation reproductive study in birds is not specifically designed to identify endocrine disruptors. If however during the study there were any microscopic or biochemical effects on the sex organs of male and female bobwhites, this did not lead to any change in reproductive performance, as determined by the number of eggs laid, egg strength, egg fertility, embryo viability, hatching rate, and chick survival. Effects on reproduction in the second generation are considered unlikely, since the endocrine effect of spirodiclofen is non-specific and the compound is only applied once per season, giving disturbed processes time to redress. Considering finally, that the findings in the mammalian toxicological and mechanistic studies were no reason for the evaluating toxicologists to ask for further data to clarify endocrine effects (e.g. special studies on effects on reproduction), there appears to be no need to ask for further data on endocrine action in birds.

2.6.2 Effects on aquatic species

Summary of acute toxicity data

In acute toxicity flow-though limit tests with technical Spirodiclofen in fish and *Daphnia magna* and in a static algal growth inhibition test, no effects were observed at the highest test concentration (0.035-0.060 mg a.s./L), which was at or near the limit of solubility of the compound in the test medium. Results of studies on the acute toxicity of Spirodiclofen, the metabolite BAJ 2740-enol and the proposed 240 SC formulation to aquatic life are summarised in Table 2.6.2-1 to 2.6.2-3.

Table 2.6.2-1. The acute toxicity of Spirodiclofen to aquatic life.

Species	Test type	LC/EC_{50} (mg/L) (95% CL)	NOEC (mg/L)
Fish			
Oncorhynchus mykiss	flow-through	>0.0351[(A)]	≥0.0351[(A)]
Lepomis macrochirus	flow-through	>0.0455[(A)]	≥0.0455[(A)]
Invertebrates			
Daphnia magna	flow-through	>0.0508[(A)]	≥0.0508[(A)]
Algae			
Pseudokirchneriella subcapitata (green alga)	static	ErC50/EbC50 >0.060[(B)]	≥0.060[(B)]

(A) Based on mean measured concentrations.
(B) Based on analytically confirmed nominal concentrations.

Table 2.6.2-2. The acute toxicity of BAJ 2740-enol to aquatic life.

Species	Test type	LC/EC_{50} (mg/L) (95% CL)	NOEC (mg/L)
Fish			
Oncorhynchus mykiss	static	>73[(A)]	≥73[(A)]
Invertebrates			
Daphnia magna	static	>100[(B)]	3.2[(B)]
Algae			
Pseudokirchneriella subcapitata (green alga)	static	ErC50 >100[(B)]; EbC50 82.8[(B)] (54.4-163)	NOErC&NOEbC 6.25[(B)]; NOEC (dens.) <3.125[(B)]

(A) Based on mean measured concentrations.
(B) Based on analytically confirmed nominal concentrations.

Table 2.6.2-3. The acute toxicity of BAJ 2740 240 SC to aquatic life.

Species	Test type	LC/EC_{50} (mg/L) (95% CL)	NOEC (mg/L)
Fish			
Oncorhynchus mykiss	static	>68.1 a.s[(A)].; >306 formn	≥68.1 a.s. [(A)]; ≥306 formn
Lepomis macrochirus	static	>58.3 a.s. [(A)]; >262 formn	≥58.3 a.s. [(A)]; ≥262 formn
Invertebrates			
Daphnia magna	static	>100 a.s. [(B)]; >450.5 formn	56 a.s. [(B)]; 252.3 formn
Algae			
Pseudokirchneriella subcapitata (green alga)	static	EbC50 & ErC50 >4.62 a.s.[(C)]; >20.8 formn	NOEbC 4.62 a.s. [(C)]; 20.8 formn NOErC&NOEC (density) 2.31 a.s. [(C)]; 10.4 formn

(A) Based on mean measured concentrations.
(B) Based on analytically confirmed nominal concentrations.
(C) Based on initial measured concentrations.

Summary of chronic toxicity data

Studies on the chronic toxicity of Spirodiclofen and the metabolite BAJ 2740-enol to aquatic life are summarised in Table 2.6.2-4 and 2.6.2-5.

Table 2.6.2-4. The chronic toxicity of Spirodiclofen to aquatic life.

Species	Type of test	NOEC (mg a.s./L)
Oncorhynchus mykiss	97-day early life stage study	0.00195[(A)]
Daphnia magna	21-day study	0.0248[(A)]
Chironomus riparius	28-day water/sediment study (emergence)	0.032

(A) Based on mean measured concentrations.
(B) Based on analytically confirmed nominal concentrations in the water.

Table 2.6.2-5. The chronic toxicity of BAJ 2740-enol to aquatic life.

Species	Type of test	NOEC (mg/L)
Oncorhynchus mykiss	97-day early life stage study	≥0.115[(A)]
Cyprinodon variegatus	115-day full fish life cycle study	≥0.190[(A)]
Daphnia magna	21-day study	32[(C)]
Chironomus riparius	28-day water/sediment study (emergence)	3.2[(B)]

(A) Based on mean measured concentrations.
(B) Based on analytically confirmed nominal concentrations in the water.
(C) Based on analytically confirmed nominal concentrations in the water.

Risk assessment (active substance)

In tests with technical Spirodiclofen, no effects were observed at the highest test concentration (0.035-0.060 mg a.s./L), which however was limited by the low solubility of the compound in the test medium (solubility in water 0.05 mg/L at pH 4 and 20°C). Acute risk assessment will therefore be based on the results of the studies with the formulated product. Based on the lowest of the available LC/EC50 values and initial PECsw values for spray drift at 3 meter, the acute TERs in Table 2.6.2-6 were calculated.

Table 2.6.2-6. Acute TERs for Spirodiclofen from spray drift at 3 m

		LC/EC50 (µg as/L)		PECsw		TER	
crop	% drift	fish	Daphnia	algae	(µg as/L)	fish	Daphnia	algae
orchard (early)	29.20	>58300	>100000	>4620	14.0	>4160	>7135	>330
orchard (late)	15.73	>58300	>100000	>4620	7.6	>7721	>13244	>612
vine (early)	2.70	>58300	>100000	>4620	0.86	>67477	>115741	>5347
vine (late)	8.02	>58300	>100000	>4620	2.6	>22717	>38965	>1800

The acute TERs for fish, *Daphnia* and algae are all far above the relevant Annex VI triggers (100, 100 and 10, respectively). Hence the acute risk from the proposed use should be low.

The long-term TERs assuming constant exposure to the initial PECsw are shown in Table 2.6.2-7.

Table 2.6.2-7. Long-term TERs for Spirodiclofen assuming constant exposure to the initial PECs

crop	distance (m)	drift (%)	NOEC (µg as/L)			PECsw (µg as/L)	TER		
			fish	Daphnia	sed. dw.		fish	Daphnia	sed. dw.
orchard (early)	3	29.20	1.95	24.8	32	14	**0.14**	**1.8**	**2.3**
	5	19.89	1.95	24.8	32	10	**0.20**	**2.6**	**3.4**
	10	11.81	1.95	24.8	32	5.7	**0.34**	**4.4**	**5.6**
	15	5.55	1.95	24.8	32	2.7	**0.73**	**9.3**	12
	20	2.77	1.95	24.8	32	1.3	**1.5**	19	24
	30	1.04	1.95	24.8	32	0.50	**3.9**	50	64
	40	0.52	1.95	24.8	32	0.25	**7.8**	99	128
	50	0.30	1.95	24.8	32	0.14	14	172	222
orchard (late)	3	15.73	1.95	24.8	32	7.6	**0.26**	**3.3**	**4.2**
	5	8.41	1.95	24.8	32	4.0	**0.48**	**6.1**	**7.9**
	10	3.60	1.95	24.8	32	1.7	**1.1**	14	19
	15	1.81	1.95	24.8	32	0.87	**2.2**	29	37
	20	1.09	1.95	24.8	32	0.52	**3.7**	47	61
	30	0.54	1.95	24.8	32	0.26	**7.5**	96	123
	40	0.32	1.95	24.8	32	0.15	13	161	208
vine (early)	3	2.70	1.95	24.8	32	0.86	**2.3**	29	37
	5	1.18	1.95	24.8	32	0.38	**5.2**	66	85
	10	0.39	1.95	24.8	32	0.12	16	199	256
vine (late)	3	8.02	1.95	24.8	32	2.6	**0.76**	9.7	12
	5	3.62	1.95	24.8	32	1.2	**1.7**	21	28
	10	1.23	1.95	24.8	32	0.39	**5.0**	63	81
	15	0.65	1.95	24.8	32	0.21	**9.4**	119	154
	20	0.42	1.95	24.8	32	0.13	15	185	238

The long-term TERs for fish, *Daphnia* and sediment dwelling organisms are above the relevant Annex VI trigger of 10 for early and late applications in orchards using buffer zones of at least 50 and 40 m, respectively, and in vine using buffer zones of at least 10 and 20 m, respectively. Hence the long-term risk from these use scenarios should be low.

For all other scenarios, the long-term TERs for fish are below the Annex VI trigger of 10. In addition, for early application in orchards, chronic TERs for *Daphnia* are below the Annex VI trigger of 10 for buffer zones of 15 m and below, and for sediment dwelling organisms for buffer zones of 10 m and below, and for late application in orchards chronic TERs for *Daphnia* and sediment dwelling organisms are below the Annex VI trigger of 10 for buffer zones of 5 m and below. Hence the risk assessment for these uses should be refined.

The study with sediment dwelling organisms was performed under static conditions, hence comparison of the NOEC with the initial PECsw is considered to be the relevant assessment procedure. The conclusion is therefore that the long-term risk for sediment dwelling organisms should be low for early and late applications in orchards using buffer zones of at least 15 and 10 m, respectively, and for applications (early and late) in vine.

NOECs for fish and Daphnia were obtained under flow-through conditions. Considering the fast dissipation of Spirodiclofen from water (max. DT50 1.1 day) and the fact that the product is applied only once per season, it may be more appropriate to compare these NOECs with the PEC TWA values rather than initial PECsw value. According to the Guidance document on Aquatic Ecotoxicology (8075/VI/97 rev 8, 2001) "*the toxicity profile of the active substance (e.g. time to onset of effects in toxicity studies), must be taken into account.*"

In Annex IIIA under point 10, the applicant argued that for *Daphnia magna* a 10-days time window would be the appropriate figure to calculate the PEC TWA values, since "*the lowest NOEC values were obtained from the endpoints "body length of parent animals" and "number of offspring/parent/reproduction day". The latter endpoint was* measured at study termination, whereas an effect on the number of new-born offspring could be measured *from study day 10 (first neonates observed) onwards*".

The applicant further argued that for fish the PEC TWA values should be calculated for a 65-days time window, since "*The growth reduction (reduced length and weight) was the selectively most sensitive endpoint in the study. Length and weight are considered as sensitive integrative endpoints for fry growth. This endpoint was measured at study termination, i.e. day 97. The period of fry growth starts after hatching from the egg on day 33 (earliest hatch) and thus the duration of this period was 65 days.*"

The long-term TERs for fish and *Daphnia magna* based on the 10-days and 65-days PEC TWA values are shown in Table 2.6.2-8.

Table 2.6.2-8. Long-term TERs of Spirodiclofen for fish and invertebrates assuming exposure to the TWA PECs 10-days (invertebrates) and 65-days (fish)

			fish			Daphnia		
crop	dis-tance (m)	drift (%)	NOEC (µg as/L)	TWA PECsw (µg as/L)	TER	NOEC (µg as/L)	TWA PECsw (µg as/L)	TER
orchard (early)	3	29.20	1.95	0.34	**5.7**	24.8	2.2	11
	5	19.89	1.95	0.23	**8.4**	24.8	1.5	16
	10	11.81	1.95	0.14	14	24.8	0.90	28
orchard (late)	3	15.73	1.95	0.18	11	24.8	1.2	21
vine (early)	3	2.70	1.95	0.021	92	24.8	0.14	181
vine (late)	3	8.02	1.95	0.063	31	24.8	0.41	61

The long-term TERs for fish and *Daphnia*, based on the 10-days and 65-days PEC TWA values, respectively, are above the Annex VI trigger of 10 for all proposed uses, except for the early application in orchards with buffer zones of 5 m or less (TERlt fish <10).

In the opinion of the Rapporteur, however, the use of a TWA concentration to calculate the TER may not be appropriate in this case as it would not take account of the initial peak exposure concentration and therefore it could potentially underestimate the risk. At the LOEC for *Daphnia magna* of 49.3 µg a.s./L, the production of offspring was already reduced for the first batch of young, produced after 10 days. The effects on growth of rainbow trout, determined at the end of the 97-day fish early life stage test, may have resulted from the exposure of the sensitive early stages during the initial study period. The applicant states in Annex IIIA under point 10, that it is "*currently developing further higher tier studies to address the risk more precisely regarding chronic effects on developing fish from the intended use of BAJ 2740 SC 240*". Similar studies could also be submitted to address the chronic effects on *Daphnia magna* under an exposure regime relevant for the practical use scenario (i.e., exposure to a pulse dose disappearing with a DT50 of about 1.1 days).

For refinement of the risk the notifier has submitted additional data. In order to address whether effects are expexted under a short period of exposure reflecting the natural conditions of a single application in a field and potential entry to a water body, a fish early life stage test was performed adapting the test design to more realistic conditions of exposure. As such, rainbow trout of the most sensitive life stage were exposed to a pulse dose of different concentrations in a water/sediment system, which resulted in a NOEC of 0.020 mg/L. This value can be used for risk assessment.

Toxicity to *Daphnia magna* was tested under flow-through conditions for 21 days. The study was required by national requirements outside the European Community and covers a similar test design as the study provided earlier. Test concentrations were analytically confirmed and a NOEC of 0.0111 mg/L was reported. Therefore, taking this endpoint into consideration, TER values need to be reexamined based on these study results. As exposure of daphnia within the toxicity tests was continuous over the whole testing period of 21 days, it can be more appropriate to relate this endpoint to a time-weighted average PEC value. The NOEC was based upon impact on reproduction as determined by the number of neonates/adult reproduction per day. As up to the maximum tested concentration of 32.7 µg a.i./L no significant differences for the time to first brood was detectable, the effects to reproduction are based on the production of neonates over the total testing period of 21 days. Hence, the TER calculations are undertaken in a refined standard approach of relating the NOEC to the PEC weighted over a time window of 21 days.

Refined risk assessment

TER calculations for fish, daphnia and sediment dwelling organisms are presented in Tables 2.6.2-9 and 2.6.2-10 for use of Spirodiclofen SC 240 in orchards and vines based upon the new endpoints given above. A refined risk assessment for daphnia is presented in Table 2.6.2-11.

Table 2.6.2-9 Long-term TERs for Fish, Daphnia and Chironomus in Orchard

Use scenario/ Crop	Distance from field [m]	PECinitial [µg/L]	Fish	NOEC [µg/L] Daphnia	Chironomus	TERlt Fish	TERlt Daphnia	Chironomus
Orchard early	3	14	20	11.1	32	**1.4**	**0.8**	**2.3**
	5	9.5	20	11.1	32	**2.1**	**1.2**	**3.4**
	10	5.7	20	11.1	32	**3.5**	**1.9**	**5.6**
	15	2.7	20	11.1	32	**7.4**	**4.1**	11.9
	20	1.3	20	11.1	32	15.4	**8.5**	24.6
	30	0.5	20	11.1	32	40.0	22.2	64.0
	40	0.25	20	11.1	32	80.0	44.4	128
	50	0.14	20	11.1	32	143	79.3	229
Use scenario/ Crop	**Distance from field [m]**	**PECinitial [µg/L]**	**Fish**	**NOEC [µg/L] Daphnia**	**Chironomus**	**Fish**	**TERlt Daphnia**	**Chironomus**
Orchard late	3	7.6	20	11.1	32	**2.6**	**1.5**	**4.2**
	5	4.0	20	11.1	32	**5.0**	**2.8**	**8.0**
	10	1.7	20	11.1	32	11.8	**6.5**	18.8
	15	0.87	20	11.1	32	23.0	12.8	36.8
	20	0.52	20	11.1	32	38.5	21.3	61.5
	30	0.26	20	11.1	32	76.9	42.7	123
	40	0.15	20	11.1	32	133.3	74.0	213
	50	0.11	20	11.1	32	181.8	101	291

Table 2.6.2-10. Long-term TERs for fish, Daphnia and Chironomus in vine

Use scenario/ Crop	Distance from field [m]	PECinitial [µg/L]	Fish	NOEC [µg/L] Daphnia	Chironomus	TERlt Fish	TERlt Daphnia	Chironomus
Vine	3	0.86	20	11.1	32	23.3	12.9	37.2
	5	0.38	20	11.1	32	52.6	29.2	84.2
	10	0.12	20	11.1	32	167	93	267
	15	0.064	20	11.1	32	313	173	500
Use scenario/ Crop	**Distance from field [m]**	**PECinitial [µg/L]**	**Fish**	**NOEC [µg/L] Daphnia**	**Chironomus**	**Fish**	**TERlt Daphnia**	**Chironomus**
Vine late	3	2.6	20	11.1	32	**7.7**	**4.3**	12.3
	5	1.2	20	11.1	32	16.7	**9.3**	26.7
	10	0.39	20	11.1	32	51.3	28.5	82.1
	15	0.21	20	11.1	32	95.2	52.9	152

The TER_{lt} obtained indicate that at a distance of 20 m and 10 m for the early and late applications in orchard, respectively, the risk to fish and Chironomus is acceptable, however, the TER for daphnia, based upon the PEC_{max} values, are below the trigger of 10 for an acceptable long term risk with these buffer zones. Hence, the risk assessment was refined as discussed earlier by consideration of the average environmental concentration (PECtwa) daphnia would be exposed to during the reproduction phase. For the use of Spirodiclofen SC 240 in vine early in season the risk to fish, daphnia and Chironomus was found to be low, as the trigger value of 10 acc. to Annex VI was met at a distance of 3 m and above from a water body. For late applications, a buffer zone of 5 m is protective for fish and Chironomus but a refinement for more realistic exposure conditions for daphnia was undertaken to demonstrate that daphnia is also not at risk for applications in vine with buffer zones of 5 m.

The results of the refined TER_{LT} for the aquatic invertebrate for all intended uses is given in the following Table.

Table 2.6.2-11 Revised Long term TERs for Daphnia in Orchards and Vine

Use scenario/ Crop	Distance from field [m]	$PEC_{21d\ twa}$ [µg/L]	Daphnia NOEC [µg/L]	TER_{lt}
Orchard early	3	1.1	11.1	10.1
	5	0.7	11.1	15.9
	10	0.43	11.1	25.8
	15	0.2	11.1	55.5
Orchard late	3	0.57	11.1	19.5
	5	0.31	11.1	35.8
	10	0.13	11.1	85.4
	15	0.07	11.1	159
Vine early	3	0.065	11.1	171
	5	0.029	11.1	383
	10	0.009	11.1	1233
	15	0.005	11.1	2220
Vine late	3	0.19	11.1	58.4
	5	0.088	11.1	126
	10	0.030	11.1	370
	15	0.016	11.1	694

The refined risk assessment for daphnia shows, that the trigger of 10 outlined in Annex VI is exceeded at a distance of 3 m for all applications. As such, the measures recommended to protect fish and Chironomus are considered sufficient to ensure that daphnia is not at an unacceptable risk.

In summary, the long term risk to fish, daphnia and sediment dwelling organisms is acceptable if buffer zones of 20 m, 10 m, 3 m and 5 m are established for uses in orchards early, late, vine early and late, respectively.

Bioaccumulation

The BCF of Spirodiclofen, normalised to 6% lipid content, was determined to be 491 L/kg for whole fish based on total radioactivity This is higher than the trigger factor of 100. However, the proposed use in orchards and vines involves a single seasonal application only. Spirodiclofen has a maximum DT50 in the water phase of 1.1 days which means that the exposure time will be relatively short, and it rapidly depurates from fish (90% clearance time of total residues in about 2 days). Besides that secondary poisoning of birds and mammals by consuming fish is not a point of concern (see 2.6.1). Bioaccumulation of Spirodiclofen is therefore not considered to be of concern. No experimentally determined BCF in fish is available for BAJ 2740-enol. The logPow value of this metabolite is 3, 0 and –2 at respective pHs 4, 7 and 9. Based on logPow = 3, the BCF of BAJ 2740-enol for fish is estimated to be 71 L/kg wet weight, according to the formula logBCF=0.85*logPow-0.7 (USES 2.0). The pH of surface water is higher than 4, hence under environmental conditions the estimated BCF of BAJ 2740-enol will be less than 71 L/kg, which is below the Annex VI trigger factor of 100. In addition, the long-term risk of BAJ 2740-enol to birds and mammals as a result of bio-accumulation, based on logPow = 3, was considered to be acceptable. Bioaccumulation of BAJ 2740-enol is therefore not considered to be of concern.

Possible endocrine effects on fish

The notifier has submitted the following statement:
Within the scope of the evaluation of the aquatic Ecotox studies on Spirodiclofen submitted to the German authorities for national registration of the compound, some questions related to the test design in general and to potential endocrine effects of the metabolite SpirodiclofenEnol (M01=BAJ 2510) were raised concerning the FFLC study of DIONNE (2001) (MO-01014409). In the following the study results are re-evaluated referring to these concerns, and the raised questions are comprehensively discussed.

1.) What was the Sex Ratio in the F0?

An evaluation of the F0 sex ratios of Cyprinodon variegatus according to the Guideline EPA § 72-5 (as cited in the final study report) is not required nor validated. An important reason for this

is that in fish a 50 : 50 sex ratio cannot be supposed to exist since various factors can have a species-specific effect to this ratio (in fish, the sex ratio is, in contrast to mammals, not genetically fixed).

However, 16 other endpoints of the F0 and the F1 generations were statistically evaluated in order to comprehensively assess potential chronic effects of the metabolite M01 (BAJ 2510) to survival, growth, and reproduction of fish. In none of the treatment groups (at mean measured concentrations of 5.7 to 190 µg/L), the metabolite caused adverse effects, therefore the report correctly states the NOEC to be 190 µg/L, and the LOEC to be > 190 µg/L.

Since a phenotypical determination of the sex in fish was done in this study during gross-pathological assessment after termination of the F0 generation at day 111 (see raw data pp. 249-276), the sex ratio of the F0 generation can, in addition to the 16 other evaluated endpoints, still be subsequently determined. The sex ratio of the F0 generation was influenced by an increased male mortality in this study (possibly an aggressive strain was used). Until the end of the F0 generation (day 111), more males than females of the fish which were maintained until they reached the stage of sexual maturity, died prematurely in the control groups (yet the validity criterion of minimum survival rates in the controls was fulfilled). Thus, in the end of the F0 generation (50 fish in total, nominally), 23 females and 14 males were in total counted in the controls (see raw data pp. 249 ff.).
Since sex ratios are binomial data, the evaluation was, in analogy to the other endpoints in this study, done by means of Fisher's Exact Binomial Test (with Bonferroni Correction) and, additionally, with the Chi Square Test (with Bonferroni Correction), respectively, using the statistics software ToxRat v. 2.08 (Ratte 2003). The analysis revealed that for the sex ratio (F0), the NOEC would be also 190 µg/L (for details, see appendix 1).

Conclusion: *This subsequent evaluation of results revealed that the sex ratio in the F0 generation seems to be not significantly influenced by the metabolite M01 up to the highest concentration tested (190 µg/L). Thus, the results of this study give no hints to endocrine effects of the metabolite M01 to fish after chronic exposure.*

2) Results of the Evaluation of the Gonads?

A histo-pathological evaluation of the gonads of C. variegatus is, according to the guideline EPA § 72-5 was not required. Moreover, such an evaluation is not validated yet for this fish species.
Consequently, only a gross evaluation of the gonads is provided in the study report (see raw data pp. 249-276). In this evaluation, however, no compound-related anomalies were found in terms of a dose-response relationship.

Conclusion: *The gross-pathological evaluation of the gonads provided in the study revealed no compound-related anomalies in the F0 generation up to the highest concentration tested (190 µg/L). Thereby, no hints to endocrine effects of the metabolite M01 can be derived from those results.*

3) According to the EPA Guideline, the Replicates During the Reproduction Phase of the Study Should Consist of Four Male and Four Female Individuals. A Justification for the Deviation from this Criterion is Missing.

According to recent EPA proposals (Detailed Review Paper (DRP) on Fish 2- Gen Tox Test / EPA Battelle, Nov 7th, 2002), two males and five females are recommended for the reproduction phase in C. variegatus. Thus, the study was in terms of this adapted to the current state of the art. This cannot be considered to be a deviation from the usual test methodology.

Conclusion: *The sex ratio chosen for the reproduction phase reflects the current state of the development of the test design.*

4) According to Which Criteria Were the 24 x 24 x 50 Eggs (i.e. at Maximum 1450 of 1555 to 2377 Laid Eggs) Selected for the Test? Randomized? Or only the Fertilized Eggs?

As it can be seen from Table 6 (study report p. 38), there were approx. 3,000 to 5,000 eggs laid in each treatment group during the reproduction phase. They were each produced in 8 to 17 distinct batches, respectively. All these batches were repeatedly (for ca 2 weeks) monitored until hatch (hatching success was around 90% in the control and in all treatment groups), and then discarded.

Only two groups of them were ***impartially*** *selected as F1 generation, to display the ELS phase*

within the test. For the selection of those two groups, rather practical considerations (batch size must be at least 50 eggs) are relevant.

Conclusion: *The eggs used for the F1 generation were impartially chosen according to practical considerations, and in compliance with the guideline for such FFLC tests. There were no hints to reduced fertilization rates, delayed development (ELS phase), or reduced survival rates in the F1 in any of the treatment groups in comparison to the control.*

Reaction RMS: With regard to the potential endocrine effects of the metabolite spirodiclofen-enol the RMS can agree with the statements and conclusions provided by the notifier. Hence, it can be concluded that there is a low potential of endocrine effects regarding the metabolite spirodiclofen-enol.

2.6.3 Effects on bees and other arthropod species

2.6.3.1 Bees

During laboratory toxicity studies technical Spirodiclofen and the 240 SC formulation were of low acute toxicity to honeybees. No effects on bees were noted at the highest test dose with the technical active substance (acute contact and oral LD50 >200 and >196 µg a.s./bee respectively) and the formulation (acute contact and oral LD50 both >100 µg a.s./bee). The hazard quotients for the orchard and vine scenarios, based on the acute oral and contact LD50 values of technical Spirodiclofen, are presented in Table 2.6.3.1.

Table 2.6.3.1. Hazard quotients for honey bees using laboratory toxicity studies on technical Spirodiclofen

crop	dose (kg a.s./ha)	oral toxicity		contact toxicity		Annex VI trigger
		LD50 (µg a.s./bee)	hazard quotient	LD50 (µg a.s./bee)	hazard quotient	
orchards	0.144	>196	<0.73	>200	<0.72	50
vine	0.096	>196	<0.49	>200	<0.48	50

The hazard ratios are well below the trigger of 50 specified in Annex VI of 91/414 EC. The acute risk to honeybees is acceptable.

Spirodiclofen reveals an IGR-related mode of action. Hence, this compound may pose a risk to honey bee brood. In a semi-field (tunnel) study, there was no adverse effect of the treatment with BAJ 2740 240 SC (dose 0.144 kg a.s./ha) on nectar and pollen collecting activity of bees, which were left in cages with treated apple trees for 6 days. Egg abundance was probably also not adversely affected, but larval and in particular pupal abundance were reduced in the BAJ 2740 240 SC treatment, and population density was also lower than in the control between day 12 and 27

post-treatment. In another trial, there was no adverse effect of the treatment with BAJ 2740 240 SC (dose 0.146 kg a.s./ha) on bee behaviour and flight intensity, mortality and colony strength of bees, which were left in cages with treated *Phacelia* for 21 days. On day 7 and 14 post-treatment however, bee brood development was reduced, with recovery being apparent at 4 weeks after treatment. These studies show an impact on bee brood development when foraging worker bees are exposed exclusively to a flowering food crop as a food source at the maximum intended rate of 145 g a.s./ha. In additional trials no evidence of an effect of BAJ 2740 240 SC at a drift rate (45 g a.s./ha) was apparent on pupal mortality and bee brood development, but the toxic standard did not give the required adequate response.In-crop effects on bees are not acceptable. An effect on bee brood, even when temporary, can have long-lasting effects on the colony since it results in reduced numbers of working bees and hence reduced foraging capacity at a later point in time. The applicant is therefore requested to submit further data to address the effects on bee brood (e.g. field tests), or to include a warning phrase for bees on the label.

Refinement of the risk

To address the risk to bee brood the notifier has proposed an appropriate labelling in order to minimize the risk to bee brood, e.g. no use of the product during flowering of the crop and avoiding that there are flowering weeds present (e.g. by mowing the weeds).

2.6.3.2 Other arthropod species

There was a comment of the UK regarding the IGR mode of action of spirodiclofen (reporting table 5(25)): *Given the IGR mode of action of spirodiclofen and the remaining uncertainty about the precise mode of toxicity/action it would be helpful to have some further clarification about the pecificity of activity. Is there for example any further information from screening studies that might be helpful in this respect?"*

Reaction RMS: The exact target molecule of spirodiclofen (acaricide, especially active against juvenile stages) has not yet been characterised (perhaps influence on molting). Screening data are presented in Vol. 3, point B.3.1.6.1. LC50 values obtained after exposure of all developmental stages on French bean leaves of the spider mite *T. urticae* ranged between 0.1 and 0.8 mg a.s./L (spray concentration). In other tests, the composite LC50 for 13 strains of *T. urticae* exposed on French bean plants was 0.33 mg a.s./L, and for 3 strains of the spider mite *P. ulmi* exposed on leaves of plum plants 0.36 mg a.s./L. For comparison, in extended laboratory tests with the predatory mite *T. pyri*, the LR50 was 2.4 g a.s./ha (exposure to treated isolated apple leaves), and >5.25 g a.s./ha (exposure of all stages to residues on apple trees). Based on the actual spray volume of 1000 and 200 L/ha, respectively, the spray concentration for the latter two LR50s corresponds to 12 and >5.25 mg a.s./L, which is a factor of at least 33 and >15 higher than the composite LC50s for spider mites. In a test with *C. carnea*, no effects were noted at the highest test dose of 144 g a.s./ha, equivalent to 720 mg a.s./L, which is a factor of at least 2000 higher than the LC50s for the target organisms.

The acute 48-hour EC50 for *Daphnia magna* is >100 mg a.s./L, indicating that adult stages of this cladoceran are not susceptible. The 21-day NOEC for *Daphnia magna* (flow-through regime) is 0.0248 mg a.s./L, but the EC50 was >0.07 mg a.s./L (highest tested concentration) for all parameters investigated. The 28-day EC50 for emergence of *C. riparius* was 0.094 mg a.s./L (static conditions). The chronic EC50 values for effects on juvenile stages of these two aquatic organisms are just below the LC50 values for the target spider mites, but it should be taken into consideration that the exposure regime for aquatic organisms (chronic exposure, fully immersed in test liquid) is worst case compared to that for target insects in screening tests (exposure to spray on plants).

More data on screening than summarised above are not available. The available data however indicate that the predatory mite *T. pyri* is 1 to 2 orders of magnitude less sensitive than the target

spider mites, the leaf dwelling *C. carnea* >3 orders of magnitude less sensitive, and that *Daphnia magna* and *C.riparius* are unlikely to be more sensitive.
This suggests that the specificity for the target organisms is high.

Also there was a comment of SE regarding the IGR mode of action of spirodiclofen (reporting table 5(26)): *Could you please confirm that the most appropriate route of uptake has been used in the terrestrial arthropod studies? Testing with other IGRs has revealed that in some cases uptake through food is a more appropriate route of uptake. That does not necessarily need to be the case with spirodiclofen but could you please confirm this.*

Reaction RMS: According to the Escort 2 guidance document testing of IGRs should be conducted with *T. pyri* and one other species (e.g. *Coccinella septempunctata, Orius laevigatus* or *Chrysoperla carnea*). For spirodiclofen testing was done with *T. pyri* and *Chrysoperla carnea*. In these tests not only mortality but also reproduction was evaluated. So, according to the available guidance the appropriate tests are available. Maybe other tests must be developed in which insects are tested by taking up food.

B.8 Environmental Fate and Behaviour

B.8.3 Predicted environmental concentrations in soil (PEC_S) (Annex IIIA 9.1.3)

PECs estimations for parent Spirodiclofen and major (>10%) metabolites were performed by the RMS using the revised DT50 values after comment by BCS.

Authorisation is requested for treatment of orchards and vines involving a single seasonal application at 0.144 and 0.096 kg a.s./ha, respectively. Initial PEC_S values were calculated under the following assumptions: 50% crop interception; homogeneous distribution in the top 5 cm layer; a soil bulk density of 1.5 g/cm^3. Initial PEC_S of the metabolites was derived from that of the a.s., with corrections for the difference in molecular mass and the maximum percentage at which the metabolite was formed from the parent substance during laboratory studies. Time weighted average (TWA) PECs values were based on worst case DT50 (lab) values. Table B.8.25 provides the substance parameters used for the calculations.

Table B.8.25. Substance parameters of Spirodiclofen and major metabolites used for PECs estimations.

parameter	Spirodiclofen	BAJ 2740-enol	BAJ 2740-ketohydroxy	BAJ 2740-dihydroxy	2,4-dichloro-benzoic acid
molecular mass	411.3	313.2	329.2	331.2	191
MMR	1	0.76	0.80	0.81	0.46
max. %	100	51.9	44.4	16.4	39.6
DT50 (d)	13	9.8	27	29.5	11

Under the above assumptions, application of BAJ 2740 240 SC will lead to the PEC_S values in Table B.8.A1 and B.8.A2.

Table B.8.A1. Initial and TWA PECs (mg/kg) of Spirodiclofen and major metabolites following application in orchards (0.144 kg a.s./ha). 50% interception

day after	Spirodiclofen		BAJ 2740-enol		BAJ 2740-ketohydroxy		BAJ 2740-dihydroxy		2,4-dichloro-benzoic acid	
appln.	Actual	TWA	actual	TWA	actual	TWA	actual	TWA	actual	TWA
0	0.096	-	0.038	-	0.034	-	0.013	-	0.018	-
1	0.091	0.093	0.035	0.037	0.032	0.033	0.012	0.013	0.017	0.017
2	0.086	0.091	0.033	0.035	0.031	0.032	0.012	0.012	0.016	0.017
4	0.078	0.086	0.029	0.033	0.028	0.031	0.012	0.012	0.014	0.016
7	0.066	0.080	0.023	0.030	0.024	0.029	0.011	0.012	0.011	0.014
21	0.031	0.058	0.009	0.020	0.012	0.021	0.008	0.010	0.005	0.010
28	0.022	0.050	0.005	0.017	0.009	0.018	0.007	0.009	0.003	0.008
50	0.007	0.034	0.001	0.010	0.003	0.013	0.004	0.007	0.001	0.005
100	0.0005	0.018	0.000	0.005	0.000	0.007	0.001	0.005	0.000	0.003

Table B.8.A2. Initial and TWA PECs (mg/kg) of Spirodiclofen and major metabolites following application in grapevine (0.096 kg a.s./ha). 50% interception

day after	Spirodiclofen		BAJ 2740-enol		BAJ 2740-ketohydroxy		BAJ 2740-dihydroxy		2,4-dichloro-benzoic acid	
appln.	actual	TWA	actual	TWA	actual	TWA	actual	TWA	actual	TWA
0	0.064	-	0.025	-	0.023	-	0.009	-	0.012	-
1	0.061	0.062	0.024	0.024	0.022	0.022	0.008	0.008	0.011	0.011
2	0.058	0.061	0.022	0.024	0.022	0.022	0.008	0.008	0.010	0.011
4	0.052	0.058	0.019	0.022	0.021	0.022	0.008	0.008	0.009	0.010
7	0.044	0.053	0.015	0.020	0.019	0.021	0.007	0.008	0.008	0.010
21	0.021	0.039	0.006	0.013	0.013	0.018	0.005	0.007	0.003	0.007
28	0.014	0.033	0.003	0.011	0.011	0.016	0.004	0.006	0.002	0.006
50	0.004	0.022	0.001	0.007	0.006	0.013	0.003	0.005	0.001	0.004
100	0.0003	0.012	0.000	0.004	0.002	0.008	0.001	0.003	0.000	0.002

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.3 Sub-chronic toxicity and reproduction toxicity (Annex IIA 8.1.3)

Bowers (2001)

Test substance	Species	Route	Duration	Criterion	Value
Spirodiclofen	*Anas platyrhynchos*	Diet	20 weeks	NOEC	734 mg/kg feed

Description

The chronic dietary toxicity, including effects on reproduction, of spirodiclofen technical (batch no. 06480/0002, purity 97.6%) to the Mallard was tested under GLP according to the EPA Guideline 71-4 and ASTM standard E 1062-86. 120 birds (60 males and 60 females) were randomly distributed among one control and 3 treatment groups. Each group consisted of 15 pens, each containing one male and one female. All birds were from the same hatch and were 23 weeks old at test initiation. Birds were fed diets containing nominal concentrations of 0, 80, 240, 720 mg a.i./kg feed (measured concentrations: 0, 79.4, 239 and 734 mg/kg feed), respectively for 20 weeks. Feed and water were provided *ad libitum*. The average temperature during the experiment in the adult room was 21 ?C, the average RH was 51%. At the beginning of week 9, the adult daily photoperiod was increased from 7 to 17 h of light to induce egg laying. This photoperiod was continued until adult termination.

Birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Adult body weights were measured on the day of study initiation (week 1), on start of weeks 3, 5, 7, 9, and at terminal sacrifice. Feed consumption was measured weekly for each pen. At end of study necropsies were performed on all of the high dose adults and at least 4 males and 4 females in the remaining treatment groups (including the controls).

Eggs were collected 1-2 times daily. Weekly eggs were counted and selected by indiscriminate draw for eggshell strength and thickness measurements. Cracked or abnormal eggs were recorded and discarded. All other eggs were first incubated at 36.3 °C, RH 55% for 3 weeks, and then transferred to a Hatcher (temperature 37.1 °C, RH 65%) for 4-5 days. Unhatched eggs per parental cage were recorded. Hatchlings were observed for 14 days. Reproductive parameters included egg production, eggshell strength/thickness, egg fertility (14-day embryo viability), 3-week embryo survival and hatchability, as well as hatchling body weight, 14-day survival and 14-day survivor body weight.

Analyses of dosing samples were performed by HPLC using UV detection.

Statistics included Chi-Square test and Levene's test of equal variance, analysis of variance (ANOVA) followed by Dunnett's test using TOXSTAT software.

Results

Actual concentrations of test diet samples collected during the test were 84.8 to 111% of nominal. Measured concentrations were 79.4, 239, and 734 mg a.i./kg feed. No test substance was detected in any of the control diets analysed.

Among adult birds no treatment related mortalities, overt signs of toxicity, or treatment related effects on body weights or feed consumption were observed at any of the concentrations tested. Necropsy revealed no apparent treatment related findings.

There were no treatment related symptoms in the hatchlings in any group. No hatchling mortalities occurred in the control or 79.4 mg/kg group; 1 and 4 mortalities were noted at 239 and 734 mg/kg, respectively. Differences in the number of 14-day survivors per hen or for 14-day-survivors as a percentage of normal hatchlings were not statistically significant. There were no significant differences ($p>0.05$; Dunnett's one-tailed test) in any of the reproductive parameters at any treatment level between the treatment groups and the control group.

Based on all parameters, including toxicity in adults and reproductive performance as well as hatchling health and survival, there were no adverse effects in mallards exposed to technical spirodiclofen in the diet up to 734 mg a.i./kg for 20 weeks. The NOEC was 734 mg a.i./kg.

Remarks by RMS

The result, a NOEC of 734 mg a.i./kg feed, based on actual measured concentrations can be used for risk assessment.

Risk assessment

Because there is already a lower NOEC-value used for risk assessment, the risk assessment will not be changed by the submission of this new study.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.2 Effects on aquatic invertebrates

B.9.2.2.2 Chronic toxicity (Annex IIA 8.2.5)

Hall and Lam (2001)

Test substance	Species	Method	Duration	pH	T [°C]	Criterion	Value
Spirodiclofen	*Daphnia magna*	Flow-through	21 d	8.0 - 8.2	20 -21	NOEC	0.0111 mg/L

Description

The chronic toxicity of spirodiclofen (batch no. 06480/0002; purity 97.8%) on survival and reproduction of *Daphnia magna* was tested according to EPA Guideline 72-4. The animals were exposed to a negative control and a solvent control (acidified methanol, 1% HCl 0.1 mol/L, 0.1 mL/L) and concentrations of 4.6, 8.3, 15.1, 27.5 and 50 µg a.i./L for 21 d in a through-flow system (6.7 x turnover rate per day). 1 L beakers with 900 mL of test solution were used to expose 10 individuals (first instars < 24 h old) in 4 replicates. Daphnids were fed with algae.

Dead organisms were counted and removed daily. Neonates were counted and removed 3 x per week. At test termination the body length of the parents and their dry weight were determined.

Data were statistically analysed by ANOVA and by Dunnett's test or Bonferroni t-test using TOXSTAT. Samples of the test solution were taken on days 0, 7, 14 and 21 and analysed directly by HPLC and UV detection.

Results

Mean measured test concentrations were 4.39 (95%), 6.65 (80%), 11.1 (73%), 20.2 (73%) and 32.7 (65%) µg/L for the 4.6, 8.3, 15.1, 27.5 and 50 µg/L nominal concentrations, respectively.

The DOC ranged from 7.4 to 9.0 mg/L. pH: 8.0 – 8.2. Temperature: 19.7 – 21.3 °C. Hardness: 162 mg $CaCO_3$ /L. Photoperiod: 16 h light, 8 h dark., 623 lx. All parameters were within acceptable ranges.

Survival observed among the treated parents was 88-100% and was not lower than in the controls. Sublethal effects (pale colouration, abnormal position and unhatched neonates) were not dose related. Reproduction appeared to be the most sensitive endpoint. There were no significant differences in the time to first brood between the solvent control and the treatment groups and for this endpoint the resulting NOEC was 32.7 µg/L. The mean number of neonates per adult was significantly affected in the 20.2 µg/L and 32.7 µg/L (measured) concentrations. The mean number of neonates per adult in the pooled controls and in the 4.39, 6.65, 11.1, 20.2 and 32.7 µg/L concentrations was 6.15, 6.01, 5.55, 5.47, 4.47 and 2.78, respectively. For this endpoint the NOEC was therefore 11.1 µg/L. For the effect on dry weight and length of the exposed adult Daphnids the NOEC was 20.2 µg/L.

Remarks by RMS

The lowest NOEC for effects of spirodiclofen on reproduction (number of offspring) of 11.1 µg a.i./L (0.0111 mg/L) actually can be used for risk assessment.

B.9.2.3 Effects on fish (Annex IIA 8.2.1; Annex IIIA 10.2.1)

B.9.2.3.3 Early life stage toxicity (Annex IIIA 10.2.2)

Dorgerloh and Sommer (2002)

Test substance	Species	Method	Duration	pH	T [°C]	Criterion	Value
Spirodiclofen	*Oncorhynchus mykiss*	Static, with sediment	42 d	6.6- 7.3	11-12	NOEC	0.020 mg/L

Description

The toxicity of spirodiclofen (batch no. 6480/0002; purity 97.1%) to early life stages (ELS) of the rainbow trout was tested according to OECD Guidelines 210 and 215. To obtain more realistic data the test was carried out as an indoor microcosm test with artificial sediment on the bottom of the test vessels. The study started 27 days post-hatching (61 d old fry). Two range finding tests showed that the 25-30 d post-hatch stage was the most sensitive ELS, based on growth effects. Fish embryos were exposed to nominal pulse concentrations of 2.5, 5.0, 10.0, 20.0 and 40.0 µg a.i./L (vehicle acetone, 0.1 mL/L) + controls. The solvent control received acetone only. Fish were kept in glass aquaria (40 L) with 33 cm water column and 2 cm sediment. The sediment was modified artificial OECD 219 soil (peat, quartz sand, kaolin clay and $CaCO_3$; pH 6.5- 8.0; 5% o.s.). There were 30 fish per concentration (initial loading: 0.124 g fish/L; initial body weight: 163-177 mg; initial length: 20.4-27.6 mm). One microcosm was used per concentration. Feeding during test occurred with brine shrimp cysts.

Observations were made on adverse effects and mortality. Mean body weight and length were measured on day 0 and at the end of the test (day 42). Growth rates were determined. Growth data were statistically analysed using the Williams test.

On days 0, 1, 3, 7 and 28 water samples were taken for direct analysis by HPLC with UV detection.

Results

The analytical determinations showed that initial measured values were 83-120% of the nominal concentrations. These rapidly decreased with an average DT_{50} value of 1.6 d in the water phase and a DT_{90} of 5.3 d.

The DOC ranged from 82% to 100%. pH: 6.6 – 7.3. Temperature: 11.0 – 12.6 °C. Hardness: 40-60 mg $CaCO_3$/L. Photoperiod: 16 h light, 8 h dark. All parameters were within acceptable ranges.

There were no adverse effects or mortality. At 40 µg/L there was a significant effect on fish wet weight, fish length and specific growth rates for wet weight. The NOEC for these parameters is therefore 20 µg/L. The specific growth rate for length in any concentration was not different from the control.

Remarks by RMS

Because of the initially measured concentrations and the rapid dissipation in the water phase the endpoint values may be based on the nominal values. The NOEC of 20 µg a.i./L (0.020 mg/L) for the chronic toxicity

of spirodiclofen to rainbow trout in a static indoor microcosm with sediment can be used for risk assessment.

Risk assessment

For refinement of the risk the notifier has submitted additional data. In order to address whether effects are expexted under a short period of exposure reflecting the natural conditions of a single application in a field and potential entry to a water body, a fish early life stage test was performed adapting the test design to more realistic conditions of exposure. As such, rainbow trout of the most sensitive life stage were exposed to a pulse dose of different concentrations in a water/sediment system, which resulted in a NOEC of 0.020 mg/L. This value can be used for risk assessment.

Toxicity to *Daphnia magna* was tested under flow-through conditions for 21 day. The study was required by national requirements outside the European Community and covers a similar test design as the study provided earlier. Test concentrations were analytically confirmed and a NOEC of 0.0111 mg/L was reported. Therefore, taking this endpoint into consideration, TER values need to be reexamined based on these study results. As exposure of daphnia within the toxicity tests was continuous over the whole testing period of 21 days, it is more appropriate to relate this endpoint to a time-weighted average PEC value. The NOEC was based upon impact on reproduction as determined by the number of neonates/adult reproduction per day. As up to the maximum tested concentration of 32.7 µg a.i./L no significant differences for the time to first brood was detectable, the effects to reproduction are based on the production of neonates over the total testing period of 21 days. Hence, the TER calculations are undertaken in a refined standard approach of relating the NOEC to the PEC weighted over a time window of 21 days.

Refined risk assessment

TER calculations for fish, daphnia and sediment dwelling organisms are presented in Tables B.9.2.3-1 and B.9.2.3-2 for use of Spirodiclofen SC 240 in orchards and vines based upon the new endpoints given above. A refined risk assessment for daphnia is presented in Table B.9.2.3-3.

Table B.9.2.3.1 Long-term TERs for Fish, Daphnia and Chironomus in Orchard

Use scenario/ Crop	Distance from field [m]	PECinitial [µg/L]	Fish	NOEC [µg/L] Daphnia	Chironomus	TERlt Fish	TERlt Daphnia	Chironomus
Orchard early	3	14	20	11.1	32	**1.4**	**0.8**	**2.3**
	5	9.5	20	11.1	32	**2.1**	**1.2**	**3.4**
	10	5.7	20	11.1	32	**3.5**	**1.9**	**5.6**
	15	2.7	20	11.1	32	**7.4**	**4.1**	11.9
	20	1.3	20	11.1	32	15.4	**8.5**	24.6
	30	0.5	20	11.1	32	40.0	22.2	64.0
	40	0.25	20	11.1	32	80.0	44.4	128
	50	0.14	20	11.1	32	143	79.3	229
Use scenario/ Crop	**Distance from field [m]**	**PECinitial [µg/L]**	**Fish**	**NOEC [µg/L] Daphnia**	**Chironomus**	**Fish**	**TERlt Daphnia**	**Chironomus**
Orchard late	3	7.6	20	11.1	32	**2.6**	**1.5**	**4.2**
	5	4.0	20	11.1	32	**5.0**	**2.8**	**8.0**
	10	1.7	20	11.1	32	11.8	**6.5**	18.8
	15	0.87	20	11.1	32	23.0	12.8	36.8
	20	0.52	20	11.1	32	38.5	21.3	61.5
	30	0.26	20	11.1	32	76.9	42.7	123
	40	0.15	20	11.1	32	133.3	74.0	213
	50	0.11	20	11.1	32	181.8	101	291

Table B.9.2.3-2 Long-term TERs for Fish, Daphnia and Chironomus in Vine

Use scenario/ Crop	Distance from field [m]	PECinitial [µg/L]	Fish	NOEC [µg/L] Daphnia	Chironomus	TERlt Fish	TERlt Daphnia	Chironomus
Vine	3	0.86	20	11.1	32	23.3	12.9	37.2
	5	0.38	20	11.1	32	52.6	29.2	84.2
	10	0.12	20	11.1	32	167	93	267
	15	0.064	20	11.1	32	313	173	500
Use scenario/ Crop	**Distance from field [m]**	**PECinitial [µg/L]**	**Fish**	**NOEC [µg/L] Daphnia**	**Chironomus**	**Fish**	**TERlt Daphnia**	**Chironomus**
Vine late	3	2.6	20	11.1	32	**7.7**	**4.3**	12.3
	5	1.2	20	11.1	32	16.7	**9.3**	26.7
	10	0.39	20	11.1	32	51.3	28.5	82.1
	15	0.21	20	11.1	32	95.2	52.9	152

The TERlt obtained indicate that at a distance of 20 m and 10 m for the early and late applications in orchard, respectively, the risk to fish and Chironomus is acceptable, however, the TER for daphnia, based upon the PECmax values, are below the trigger of 10 for an acceptable long term risk with these buffer zones. Hence, the risk assessment was refined as discussed above by consideration of the average environmental concentration (PECtwa) daphnia would be exposed to during the reproduction phase. For the use of Spirodiclofen SC 240 in vine early in season the risk to fish, daphnia and Chironomus was found to be low, as the trigger value of 10 acc. to Annex VI was met at a distance of 3 m and above from a water body. For late applications, a buffer zone of 5 m is protective for fish and Chironomus but a refinement for more realistic exposure conditions for daphnia was undertaken to demonstrate that daphnia is also not at risk for applications in vine with buffer zones of 5 m.

The results of the refined TERLT for the aquatic invertebrate for all intended uses is given in the following Table.

Table B.9.2.3-3 Revised Long term TERs for Daphnia in Orchards and Vine

Use scenario/ Crop	Distance from field [m]	$PEC_{21d\ twa}$ [µg/L]	Daphnia NOEC [µg/L]	TER_{lt}
Orchard early	3	1.1	11.1	10.1
	5	0.7	11.1	15.9
	10	0.43	11.1	25.8
	15	0.2	11.1	55.5
Orchard late	3	0.57	11.1	19.5
	5	0.31	11.1	35.8
	10	0.13	11.1	85.4
	15	0.07	11.1	159
Vine early	3	0.065	11.1	171
	5	0.029	11.1	383
	10	0.009	11.1	1233
	15	0.005	11.1	2220
Vine late	3	0.19	11.1	58.4
	5	0.088	11.1	126
	10	0.030	11.1	370
	15	0.016	11.1	694

The refined risk assessment for daphnia shows, that the trigger of 10 outlined in Annex VI is exceeded at a distance of 3 m for all applications. As such, the measures recommended to protect fish and Chironomus are considered sufficient to ensure that daphnia is not at an unacceptable risk.

In summary, the long term risk to fish, daphnia and sediment dwelling organisms is acceptable if buffer zones of 20 m, 10 m, 3 m and 5 m are established for uses in orchards early, late, vine early and late, respectively.

B.9.11 New references relied on

Annex point	Author	Year	Title	Date	Owner

				protection claimed	
IIA 8.1.3	Bowers, L.M.	2001	Effect of technical BAJ 2740 on mallard reproduction. Report no. 110591. MO-03-005295	Yes	Bayer Crop Sciences
IIIA 10.2.2	Dorgerloh, M. and H. Sommer	2002	Chronic effects of BAJ 2740 on selected early life stages of rainbow trout (*Oncorhynchus mykiss*) under more realistic conditions of exposure. [redacted] Report DOM 20044. MO-02-014087	Yes	Bayer Crop Sciences
IIA 8.2.5	Hall, A.T. and C.V. Lam	2001	Chronic toxicity of BAJ 2740 technical to the waterflea (*Daphnia magna*) under flow-through conditions. [redacted] Report no. 110095. MO-01-014192	Yes	Bayer Crop Sciences

Appendix 1 (Details of analysis, based on ToxRat version 2.08)

Statistical Evaluation of a Quantal Response: FFLC #110096 Springborn-Report

General:

Test identification/project no.	Fflc #110096 springborn-report
Test material	Baj 2510
Unit of test material concentration	µg/l
Date and time of the evaluation	04.02.2004; 16:00:02
Raw data filename:	sex ratio_BAJ2510.xls
Number of treatments (incl. control(s))	7
Duration of the test	111 d
Measurement variable	#females/ sex ratio
Test system	Sheepshead minnow

Relation of #females/ sex ratio on Concentration

#females/ sex ratio of Sheepshead minnow as Dependent on Concentration

Tab. 1: #females/ sex ratio of Sheepshead minnow as dependent on concentration of the test item; Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation

Treatm. [µg/L]	**control**	**5,7**	**13,0**	**24,0**	**48,0**	**110,0**	**190,0**
repl A **(all** sexes)	19	12	24	22	20	24	17
repl B **(all** sexes)	18	17	24	25	24	16	20
Total:	37	29	48	47	44	40	37
n:	2	2	2	2	2	2	2
female:							
repl A	10	6	16	15	13	19	13
repl B	13	10	16	19	14	12	17
Total	23	16	32	34	27	31	30
n:	2	2	2	2	2	2	2

Fisher`s Exact Binomial Test with Bonferroni Correction

Tab. 2: Fisher`s Exact Binomial Test with Bonferroni Correction: Pair-wise comparisons between treatment and control on the multiple significance level (alpha is 0,05; onesided greater). Pair-wise comparisons are performed sequentially using the adjusted Alpha* (= alpha/(k-1); k: number of comparisons (after Holm 1979)); Ho (no effect)is accepted, if the probability p > Alpha*.

Treatm.[µg/L]	all sexes	female	male	ratio	p	alpha*	sign.
control	37	23	14	37,8			
5,7	29	16	13	44,8	0,009	0,008	-
13,0	48	32	16	33,3	0,419	0,025	-
24,0	47	34	13	27,7	0,224	0,017	-
48,0	44	27	17	38,6	1,000	0,050	-
110,0	40	31	9	22,5	0,111	0,010	-
190,0	37	30	7	18,9	0,121	0,013	-

The prerequisites for the corresponding Chi²-test are fulfilled. If the exact test fails, a Chi²-test may be conducted.

Chi² 2x2 Table

Tab. 2: Chi²-2x2 Test (alpha is 0,05; one-sided greater): Pair-wise comparisons between treatment and control; The one-sided test is based on the standard normal variable z, which is a measure of the difference between treatment and control; Ho (no effect) is accepted, if the probability p(z) > Alpha.

Treatm.[µg/L]	all sexes	female	male	ratio	z	p(z)sign.
control	37	23	14	37,8		
5,7	29	16	13	44,8	0,321	0,374 -
13,0	48	32	16	33,3	0,202	0,420 -
24,0	47	34	13	27,7	0,756	0,225 -
48,0	44	27	17	38,6	0,156	0,438 -
110,0	40	31	9	22,5	1,220	0,111 -
190,0	37	30	7	18,9	1,547	0,061 -

European Commission

SPIRODICLOFEN

ADDENDUM

VOLUME 4

ANNEX C

Rapporteur Member State: The Netherlands

June 2005, revised September 2006

Draft Assessment Report and Proposed Decision of The Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

B.6.13 DERMAL ABSORPTION

In the DAR, the proposed dermal absorption value was 2% for both concentrate and diluted spirodiclofen, based on an *in vivo* study in male monkeys.

The experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. A number of experts indicated that they would not have accepted the study initially, particularly as there were clear OECD guidelines on both *in vivo* and *in vitro* assessment of dermal absorption. The experts discussed the proposed dermal absorption value of 2%. Areas of concern with the monkey study included the fact that levels of radioactivity in the skin and body were not determined, and the level of total radioactivity recovered (92%). The low level of variation in individual animals supported the theory that the 8% of "lost" radioactivity may have been absorbed, and thus the experts concluded that this should be incorporated into the dermal absorption to give a value of 10%.
Experts considered the physical chemical properties of spirodiclofen, and considered that the molecular weight and K_{OW} supported a dermal absorption value of 10%. It was therefore concluded that the dermal absorption be set at a value of 10% for the concentrate, based on physicochemical properties and supported by the studies in monkeys.
It was additionally noted that no data was available on the dermal absorption potential of the formulation dilution. Therefore a value of 65% was proposed, based on the oral absorption value.
However, a re-assessment by the RMS after the EPCO meeting revealed that these conclusions were drawn on wrong assumptions, and this was further explained in the revised addendum of September 2006. In summary, the RMS claims that the concentration as tested in the in vivo study was a rather low area dose and should be considered an acceptable area dose to be used for the spray concentration. Hence the RMS now proposes a dermal absorption value of 10% for the concentrate and spray dilution. This is also more in line with the general practice of applying one default value for both the concentrate and the spray dilution. Moreover, the applicant still is of the opinion that a dermal absorption for concentrate and spray dilution of ca 2% is justified, based on the results of an exploratory study in monkeys. Since at this stage this study cannot be accepted anymore in the Annex-I procedure, the RMS proposes that the acceptability of the exploratory study, and a possible lowering of the proposed dermal absorption of 10% to ca. 2% will be a Member State issue after the Annex I inclusion.

B.6.14 EXPOSURE DATA

The exposure data below are calculated with the dermal absorption values of 10% (concentrate) and 65% (dilution), as agreed upon during the Expert meeting. As explained above and in the final addendum, these values were drawn on wrong assumptions, and this was further explained in the revised addendum of September 2006. The calculation with the

correct values of 10% for both concentrate and dilution were presented in the final addendum of September 2006.

B.6.14.2 Exposure and risk assessment

Internal exposures and risks as % AOEL are specified in Table 6.14.2.1, 6.14.2.2 and 6.14.2.3, based on dermal absorption values of 10% (concentrate) and 65% (dilution).

Table 6.14.2.1 Operator internal exposure and risk assessment

Model	Estimated internal exposure (mg a.s./day)		AOEL-systemic[1] (mg a.s./day)	% AOEL	
	without PPE	with PPE		without PPE	with PPE
Mechanical upward spraying in grapes					
UK- 75th	9.03	5.78	0.54	1672	1070
DE- GM	5.94	0.56	0.63	943	89
Mechanical upward spraying in pome fruits, and stone fruits					
UK- 75th	9.75	5.56	0.54	1806	1030
DE- GM	8.91	0.84	0.63	1414	133
Mechanical upward spraying in citrus					
UK- 75th	5.96	2.89	0.54	1104	535
DE- GM	8.91	0.84	0.63	1414	133
Manual upward spraying in grapes					
UK- 75th	12.9	3.75	0.54	2389	694
DE- GM	2.04	0.13	0.63	324	21
Manual upward spraying in pome fruits, and stone fruits					
UK- 75th	12.9	3.75	0.54	2389	694
DE- GM	3.05	0.19	0.63	484	30
Manual upward spraying in citrus					
UK- 75th	9.67	2.2	0.54	1791	407
DE- GM	3.05	0.19	0.63	484	30

[1] The AOEL systemic is 0.54 mg/day, using a body weight of 60 kg for the UK-model, and 0.63 mg/day, using a body weight of 70 kg for the German model.

Table 6.14.2.2 Bystander internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic (mg a.s./day)	%AOEL
Exposure during mechanical upward spraying on grapes			
Total exposure	**0.63**	**0.63**	**100**
Exposure during mechanical upward spraying on pome/stone fruit, citrus			
Total exposure	**0.94**	**0.63**	**150**

Table 6.14.2.3 Worker internal exposure and risk assessment

Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic	% AOEL	
	without PPE	with PPE	(mg a.s./day)	without PPE	with PPE
Exposure after mechanical upward spraying on grapes					
Total exposure	8.424	0.842	0.63	1337	134
Exposure after mechanical upward spraying on pome/stone fruit, citrus					
Total exposure	12.636	1.264	0.63	2006	201

- The inhalation exposure is not quantifiable with this method.

Conclusion

In many cases no safe use is calculated for operator, bystander and worker, when exposure data are calculated with the dermal absorption values of 10% (concentrate) and 65% (dilution), as agreed upon during the Expert meeting. However, as explained above (B.6.13) and in the final addendum, these values were drawn on wrong assumptions, and this was further explained in the revised addendum of September 2006. The calculation with the more correct values of 10% for both concentrate and dilution were presented in the final addendum of September 2006.

APPENDIX 1: Exposure model calculations and risk assessment for all applications

1. BASIC APPLICATION INFORMATION

1.4 RISK ASSESSMENT

Input data

Dermal absorption	:	10 % (concentrate), 65% (dilution)
Inhalation absorption	:	100 %

2. OPERATOR EXPOSURE

2.1 External exposure estimates with the UK model

2.1.1 Mechanical upward spraying grapes without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**BJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.4**	l/ha	Work rate/day	**15**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0.06	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.06	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.06	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	14.4	mg/day	11.6352	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	1.44	mg/day	7.56288	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	9.03168	mg/day
Operator body weight	60	kg
Operator exposure	0.150528	mg/kg bw/day

2.1.2 Mechanical upward spraying in grapes with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	**BJ 2740 SC 240**	Active substance	**spirodiclofen**	
Formulation type	organic solvent-based	a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10** %	Dermal absorption from spray	**65**	%
Container	1 litre any closure			
PPE during mix/loading	Gloves	PPE during application	Gloves	
Dose	**0.4** l/ha	Work rate/day	**15**	ha
Application volume	**1000** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0.06	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.006	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.006	ml/day	85.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	1.44	mg/day	8.1792	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.144	mg/day	5.31648	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	5.48928	mg/day
Operator body weight	60	kg
Operator exposure	0.091488	mg/kg bw/day

2.1.3 Mechanical upward spraying in pome fruits/stone fruits without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**BJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.09	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	21.6	mg/day	11.6352	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	2.16	mg/day	7.56288	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	9.75168	mg/day
Operator body weight	60	kg
Operator exposure	0.162528	mg/kg bw/day

2.1.4 Mechanical upward spraying in pome fruits/stone fruits with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**BJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.009	ml/day	85.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	2.16	mg/day	8.1792	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.216	mg/day	5.31648	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	5.56128	mg/day
Operator body weight	60	kg
Operator exposure	0.092688	mg/kg bw/day

2.1.5 Mechanical upward spraying in citrus without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**BJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.09	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	21.6	mg/day	5.8176	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	2.16	mg/day	3.78144	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.0144	mg/day
Percent absorbed	100	%
Absorbed dose	0.0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	5.95584	mg/day
Operator body weight	60	kg
Operator exposure	0.099264	mg/kg bw/day

2.1.6 Mechanical upward spraying in citrus with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**BJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	2%	5%	
Dermal exposure	4	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.009	ml/day	85.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	2.16	mg/day	4.0896	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.216	mg/day	2.65824	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.0144	mg/day
Percent absorbed	100	%
Absorbed dose	0.0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	2.88864	mg/day
Operator body weight	60	kg
Operator exposure	0.048144	mg/kg bw/day

2.1.7 Manual upward spraying in grapes without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.4**	l/ha	Work rate/day	**0.4**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	0.4	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	64.8	mg/day	9.792	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	6.48	mg/day	6.3648	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	12.85632	mg/day
Operator body weight	60	kg
Operator exposure	0.214272	mg/kg bw/day

2.1.8 Manual upward spraying in grapes with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.4**	l/ha	Work rate/day	**0.4**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	0.4	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1.25	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49.5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	49.5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	6.48	mg/day	4.752	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.648	mg/day	3.0888	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	3.74832	mg/day
Operator body weight	60	kg
Operator exposure	0.062472	mg/kg bw/day

2.1.9 Manual upward spraying in pome fruits, stone fruits, without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**0.267**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.266666667	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	64.8	mg/day	9.792	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	6.48	mg/day	6.3648	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	12.85632	mg/day
Operator body weight	60	kg
Operator exposure	0.214272	mg/kg bw/day

2.1.10 Manual upward spraying in pome fruits, stone fruits, with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.6**	l/ha	Work rate/day	**0.267**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.266666667	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1.25	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49.5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	49.5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	6.48	mg/day	4.752	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.648	mg/day	3.0888	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	3.74832	mg/day
Operator body weight	60	kg
Operator exposure	0.062472	mg/kg bw/day

2.1.11 Manual upward spraying in citrus without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**0.133**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.133333333	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	64.8	mg/day	4.896	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	6.48	mg/day	3.1824	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.00576	mg/day
Percent absorbed	100	%
Absorbed dose	0.00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	9.66816	mg/day
Operator body weight	60	kg
Operator exposure	0.161136	mg/kg bw/day

2.1.12 Manual upward spraying in citrus with PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**BAJ 2740 SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**10**	%	Dermal absorption from spray	**65**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	Gloves	
Dose	**0.6**	l/ha	Work rate/day	**0.133**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.133333333	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	20%	18%	
Dermal exposure	1.25	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	49.5	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	49.5	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	6.48	mg/day	2.376	mg/day
Percent absorbed	10	%	65	%
Absorbed dose	0.648	mg/day	1.5444	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.00576	mg/day
Percent absorbed	100	%
Absorbed dose	0.00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	2.19816	mg/day
Operator body weight	60	kg
Operator exposure	0.036636	mg/kg bw/day

2.2 External exposure estimates with the German model

The German model is based on unpublished studies performed by industry and all carried out in Germany. For mixing/loading the nature of the formulation is an important variable. The format of exposure is mg/kg and the chosen statistic is the geometric mean (GM).

2.2.1 Mechanical upward spraying in grapes

= HIGH_CROP_TRACTOR_MOUNTED =

Treated area per day	**A =**	**8**	**ha/d**	at BBA = 8
Use rate	**R =**	***0.096***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0.0006	0.07	0.008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0.0006***	0.096	8	0.0004608	mg/pers. x d
Ia = I*a x R x A	0.018	0.096	8	0.013824	mg/pers. x d
		I, in total =		**0.0142848**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.096	8	1.8432	mg/pers. x d
Da/h = D*a/h x R x A	0.7	0.096	8	0.5376	mg/pers. x d
Da/c = D*a/c x R x A	1.2	0.096	8	0.9216	mg/pers. x d
Da/b = D*a/b x R x A	9.6	0.096	8	7.3728	mg/pers. x d
		D, in total =		**10.6752**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**			
Im =	0.0004608	-	1		0.0004608	mg/pers. x d
Ia =	0.013824	-	1		0.013824	mg/pers. x d
					0.0142848	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	1.8432	SS 110	0.01		0.018432	mg/pers. x d
Da/h =	0.5376	SS 120	0.01		0.005376	mg/pers. x d
Da/c =	0.9216	SS 420	0.5		0.4608	mg/pers. x d
Da/b =	7.3728	SS 220	0.05		0.36864	mg/pers. x d
					0.853248	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0004608	0.0004608	0.0004608	0.0004608
Inhalation: appl.	100%	0.013824	0.013824	0.013824	0.013824
Dermal: m/l	10%	1.8432	0.018432	0.18432	0.0018432
Dermal: appl.	65%	8.832	0.834816	5.7408	0.5426304
			mg/pers./d:	5.9394048	0.5587584
kg bw:	70		mg/kg bw/d:	0.08484864	0.00798226
syst. AOEL:	0.009		**% of AOEL:**	**942.762667**	**88.6918095**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

2.2.2 Manual upward spraying in grapes

For manual spraying with a 15L knapsack, a treated area of 0.15-0.45 ha is used. It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.

= HIGH_CROP_HAND_HELD (HCHH) = **(at BBA)**

Treated area per day	**A =**	***0.45***	**ha/d**	**(1 ha/d)**
Use rate	**R =**	***0.096***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	I*a = 0,3	D*a/c = 4,8
I*m	0.05	0.8	0.02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = I*m x R x A	***0.05***	0.096	0.45	0.00216 mg/pers. x d
Ia = I*a x R x A	0.3	0.096	0.45	0.01296 mg/pers. x d
		I, in total =		**0.01512 mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0.096	0.45	8.856 mg/pers. x d
Da/h = D*a/h x R x A	10.6	0.096	0.45	0.45792 mg/pers. x d
Da/c = D*a/c x R x A	4.8	0.096	0.45	0.20736 mg/pers. x d
Da/b = D*a/b x R x A	25	0.096	0.45	1.08 mg/pers. x d
		D, in total =		**10.60128 mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.00216	-	1		0.00216 mg/pers. x d
Ia =	0.01296	-	1		0.01296 mg/pers. x d
					0.01512 mg/pers. x d

Estimated derm. exp.

Dm/h =	8.856	SS 110	0.01		0.08856 mg/pers. x d
Da/h =	0.45792	SS 120	0.01		0.0045792 mg/pers. x d
Da/c =	0.20736	SS 420	0.5		0.10368 mg/pers. x d
Da/b =	1.08	SS 220	0.05		0.054 mg/pers. x d
					0.2508192 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.00216	0.00216	0.00216	0.00216
Inhalation: appl.	100%	0.01296	0.01296	0.01296	0.01296
Dermal: m/l	10%	8.856	0.08856	0.8856	0.008856
Dermal: appl.	65%	1.74528	0.1622592	1.134432	0.10546848
			mg/pers./d:	2.035152	0.12944448
kg bw:	70		mg/kg bw/d:	0.0290736	0.00184921
syst. AOEL:	0.009		**% of AOEL:**	**323.04**	**20.5467429**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

2.2.3 Mechanical upward spraying in pome fruits, stone fruits and citrus

= HIGH_CROP_TRACTOR_MOUNTED =

Treated area per day	**A =**	**8**	**ha/d**	at BBA = 8
Use rate	**R =**	***0.144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0.0006	0.07	0.008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0.0006***	0.144	8	0.0006912	mg/pers. x d
Ia = I*a x R x A	0.018	0.144	8	0.020736	mg/pers. x d
		I, in total =		**0.0214272**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.144	8	2.7648	mg/pers. x d
Da/h = D*a/h x R x A	0.7	0.144	8	0.8064	mg/pers. x d
Da/c = D*a/c x R x A	1.2	0.144	8	1.3824	mg/pers. x d
Da/b = D*a/b x R x A	9.6	0.144	8	11.0592	mg/pers. x d
		D, in total =		**16.0128**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.0006912	-	1		0.0006912 mg/pers. x d
Ia =	0.020736	-	1		0.020736 mg/pers. x d
					0.0214272 mg/pers. x d

Estimated derm. exp.

Dm/h =	2.7648	SS 110	0.01		0.027648 mg/pers. x d
Da/h =	0.8064	SS 120	0.01		0.008064 mg/pers. x d
Da/c =	1.3824	SS 420	0.5		0.6912 mg/pers. x d
Da/b =	11.0592	SS 220	0.05		0.55296 mg/pers. x d
					1.279872 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0006912	0.0006912	0.0006912	0.0006912
Inhalation: appl.	100%	0.020736	0.020736	0.020736	0.020736
Dermal: m/l	10%	2.7648	0.027648	0.27648	0.0027648
Dermal: appl.	65%	13.248	1.252224	8.6112	0.8139456
			mg/pers./d:	8.9091072	0.8381376
kg bw:	70		mg/kg bw/d:	0.12727296	0.01197339
syst. AOEL:	0.009		**% of AOEL:**	**1414.144**	**133.037714**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	

Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

2.2.4 Manual upward spraying in pome fruits, stone fruits, and citrus

For manual spraying with a 15L knapsack, a treated area of 0.15-0.45 ha is used. It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.

= HIGH_CROP_HAND_HELD (HCHH) = **(at BBA)**

	Treated area per day	**A =**	***0.45***	**ha/d**	**(1 ha/d)**
	Use rate	**R =**	***0.144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	l*a = 0,3	D*a/c = 4,8
l*m	0.05	0.8	0.02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = l*m x R x A	***0.05***	0.144	0.45	0.00324	mg/pers. x d
Ia = l*a x R x A	0.3	0.144	0.45	0.01944	mg/pers. x d
		I, in total =		**0.02268**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0.144	0.45	13.284	mg/pers. x d
Da/h = D*a/h x R x A	10.6	0.144	0.45	0.68688	mg/pers. x d
Da/c = D*a/c x R x A	4.8	0.144	0.45	0.31104	mg/pers. x d
Da/b = D*a/b x R x A	25	0.144	0.45	1.62	mg/pers. x d
		D, in total =		**15.90192**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**			
Im =	0.00324	-	1		0.00324	mg/pers. x d
Ia =	0.01944	-	1		0.01944	mg/pers. x d
					0.02268	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	13.284	SS 110	0.01		0.13284	mg/pers. x d
Da/h =	0.68688	SS 120	0.01		0.0068688	mg/pers. x d
Da/c =	0.31104	SS 420	0.5		0.15552	mg/pers. x d
Da/b =	1.62	SS 220	0.05		0.081	mg/pers. x d
					0.3762288	**mg/pers. x d**

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.00324	0.00324	0.00324	0.00324
Inhalation: appl.	100%	0.01944	0.01944	0.01944	0.01944
Dermal: m/l	10%	13.284	0.13284	1.3284	0.013284
Dermal: appl.	65%	2.61792	0.2433888	1.701648	0.15820272
			mg/pers./d:	3.052728	0.19416672
kg bw:	70		mg/kg bw/d:	0.0436104	0.00277381
syst. AOEL:	0.009		**% of AOEL:**	**484.56**	**30.8201143**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

3. Bystander exposure

3.2 Exposure estimates

Mechanical upward spraying in grapes

BYSTANDER EXPOSURE			**EUROPOEM MODEL**	
	BAJ 2740 SC 240		**Outdoor application**	
as	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.096	kg a.s. / ha	summary of intended uses
SV	Spray volume	1000	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.06	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0072**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.05		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**0.96**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IF	Inhalation Absorption Fraction	1		
DF	Dermal Absorption Fraction	0.65		
	AOEL	0.63	mg a.s./ day	
		Without PPE		
	Internal exposure	[mg a.s./ day]		
	Inhalation	0.0072		IE(int) = IE x IF
	Dermal	0.624		DE(int) = DE x DF
	Total	**0.631**		**sum**
	% AOEL			
	Inhalation	1.1		%AOEL = 100 x IE(int) / AOEL
	Dermal	99.0		%AOEL = 100 x DE(int) / AOEL
	Total	**100**		**sum**

Mechanical upward spraying in citrus, pome fruits, and stone fruits

BYSTANDER EXPOSURE — EUROPOEM MODEL

BAJ 2740 SC 240 as spirodiclofen

Outdoor application

Parameter		Value	Unit	References, comments
SPRAYING Process outdoor				
AR	Application rate	0.144	kg a.s. / ha	summary of intended uses
SV	Spray volume	1500	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.06	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0072**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.05		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**1.44**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IF	Inhalation Absorption Fraction	1		
DF	Dermal Absorption Fraction	0.65		
	AOEL	0.63	mg a.s./ day	

	Without PPE	
Internal exposure	[mg a.s./ day]	
Inhalation	0.0072	IE(int) = IE x IF
Dermal	0.936	DE(int) = DE x DF
Total	**0.943**	**sum**
% AOEL		
Inhalation	1.1	%AOEL = 100 x IE(int) / AOEL
Dermal	148.6	%AOEL = 100 x DE(int) / AOEL
Total	**150**	**sum**

4. Worker exposure

4.3 Worker exposure estimations

Mechanical upward spraying in grapes

WORKER EXPOSURE			**EUROPOEM MODEL**	
	BAJ 2740 SC 240		**Re-entry in the field, DFR model**	
as	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.096	kg a.s./ha	summary of intended uses
Worker				
Duration				
Tc	Cutting	3	hour	Dutch model
Tsb	Sorting/ bundling	3	hour	Dutch model
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
LAI	Leaf area index (surrogate value)	2	m2/ m2	Europoem II
DFR	Dislodgeable foliar residue	4.8	mg a.s./m2	DFR = AR X 100 / LAI
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**12.96**	mg a.s./ day	DE = DFR x TC x (Tc+Tsb)
Internal exposure				
DF	Dermal Absorption Fraction	0.65		
	PPE-factor dermal	0.1		reduction factor
	AOEL	0.63	mg a.s./ day	

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	8.424	0.842	DE(int) = DE x DF
Total	**8.424**	**0.842**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	1337	134	%AOEL = 100 x DE(int) / AOEL
Total	**1337**	**134**	**sum**

Mechanical upward spraying in citrus, pome fruits, and stone fruits

WORKER EXPOSURE			**EUROPOEM MODEL**	
	BAJ 2740 SC 240		**Re-entry in the field, DFR model**	
as	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.144	kg a.s./ha	summary of intended uses
Worker				
Duration				
Tc	Cutting	3	hour	Dutch model
Tsb	Sorting/ bundling	3	hour	Dutch model
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
LAI	Leaf area index (surrogate value)	2	m2/ m2	Europoem II
DFR	Dislodgeable foliar residue	7.2	mg a.s./m2	DFR = AR X 100 / LAI
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**19.44**	mg a.s./ day	DE = DFR x TC x (Tc+Tsb)
Internal exposure				
DF	Dermal Absorption Fraction	0.65		
	PPE-factor dermal	0.1		reduction factor
	AOEL	0.63	mg a.s./ day	

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	12.636	1.264	DE(int) = DE x DF
Total	**12.636**	**1.264**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	2006	201	%AOEL = 100 x DE(int) / AOEL
Total	**2006**	**201**	**sum**

Discussion of the results

The estimated exposures must be considered relevant for the crops with the highest levels of contact with the crop and thus highest levels of exposure. The results for worker exposure are considered to reflect a worst case situation. Exposure levels will be lower for crops with only minor contact between crop and worker during re-entry activities or with increased PHI. The exposure levels are upper bound for another reason; dissipation is considered not to occur between application and crop activities.

In the GAP information, it is stated that BAJ 2740 SC 240 should be applied only once during the grow season. Thus, worker exposure will probably be limited to a short period of re-entry tasks shortly after application.

European Commission

SPIRODICLOFEN

ADDENDUM

VOLUME 3 (B6)

ANNEX B

Rapporteur Member State: The Netherlands

APRIL 2009

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

CONTENTS

Introduction

For spirodiclofen, the peer review was in principle finished in June 2007, see the EFSA conclusion in the EFSA Scientific Report (2007) 104, 1-85. However, the Commission has not sent spirodiclofen to the SCFCAH, because Annex I inclusion could not be proposed (and since spirodiclofen is a new active substance, noninclusion is not an option). The Commission therefore asked EFSA to reconsider the open points. For mammalian toxicology, there were no open points, data requirements or data gaps after the expert meeting (EPCO 28, d.d. 27 June – 1 July 2005) and evaluation meeting (d.d. 4-6.12.2006), see evaluation table rev. 2-1 (d.d. 20.12.2006). However, there was no safe use for the bystander and worker, see EFSA conclusion.

Furthermore, after the expert meeting the RMS has evaluated additional information (with regard to full scale batches and a DNT study), but this has not yet been peer reviewed. Finally, the RMS recently received new information (a supplementary DNT study and new dermal absorption data).

In summary, the points that should be considered for mammalian toxicology in the PRAPeR 69 meeting (4-8 May 2009):

- Full scale production batches. In the DAR the production process of pilot batches and the pilot plant specification have been evaluated. At the end of the peer review process, information became available on the full scale production batches. The full scale production resulted in three new impurities. For mammalian toxicology, an evaluation of the full scale specification has already been performed, but this has not yet been peer reviewed. See the final addendum from November 2006, and specifically the Chapter with the revised addendum B6 B7 from September 2006, B.6.8.1.3 (Toxicological assessment of the new proposed specification), page 56-58.
- Developmental neurotoxicity study (DNT study). In the final addendum from November 2006, in the Chapter with the revised addendum B6 B7 from September 2006, a DNT study has been evaluated, but this has not yet been peer reviewed. See B.6.7 (neurotoxicity), page 49-53.
- Supplementary DNT study. In order to address specific questions by EPA and PMRA, the notifier repeated parts of the DNT study. The RMS recently received the new study (d.d. 01-08-2008) which is evaluated in this addendum.

- Dermal absorption. The RMS recently received new dermal absorption data (d.d. 26.11.2008) which is evaluated in this addendum.
- Risk assessment. Because new dermal absorption values are proposed based on the new dermal absorption data, a revised risk assessment is performed in this addendum.

B.6 TOXICOLOGY AND METABOLISM

B.6.7 NEUROTOXICITY

B.6.7.4 Developmental neurotoxicity

In the final addendum from November 2006, in the Chapter with the revised addendum from September 2006, a DNT study has been evaluated, but this has not yet been peer reviewed. See B.6.7 (neurotoxicity), page 49-53 of the final addendum (November 2006).

The study was considered acceptable and a NOAELmaternal and developmental of 32 mg/kg bw/day was proposed by the RMS. Neurotoxic effects were not observed.
However, as also described in the addendum from September 2006, the notifier also submitted this DNT study to the US-EPA and Canada's PMRA for an application for the registration of spirodiclofen in the USA and Canada. EPA and PMRA concluded that the compound showed an effect on memory retention in rats as measured in a water maze test, hence no NOAEL could be determined. Moreover, it was concluded that a treatment-related change in brain morphometric parameters occurred in the rats subjected to this test. These conclusions were taken into account during the evaluation of the DNT study by the RMS.

In order to address specific questions by EPA and PMRA, the notifier repeated parts of the DNT study. This supplementary DNT study is evaluated and summarized below.

Characteristics of study:

Reference	:	Gilmore *et al.*, 2007	Route	:	oral, diet
Year of execution	:	2006	Group size	:	30 females/dose
Guideline	:	OPPTS 870.6300 (EPA, 1998)	Exposure	:	gestation day (GD) 0 to lactation day (LD) 21 (dams) to post-natal day 21 (offspring)
GLP	:	yes	Doses	:	0, 70, 350, 1500 mg/kg food[1]
Test substance	:	Spirodiclofen, batch no. 06480/0002; beige powder	Vehicle	:	diet

Purity	:	98.3%	Acceptable	:	yes, as supplementary study
Species	:	rat, Wistar Hannover Crl:WI	**NOAEL**mat	:	n/a (1500 mg/kg food (119 mg/kg bw/d)[2]
			NOAELdev	:	n/a (350 mg/kg food (29 mg/kg bw/d)[2]
			neurotoxic effects	:	not observed

[1] equal to 0, 5.3, 29, 119 mg/kg bw/d (gestation) and 0, 13, 66 and 263 mg/kg bw/d (lactation)

[2] due to the limited number of parameters investigated no NOAELs to be used in risk assessment may be derived based only on this study

STUDY DESIGN

The study was set-up to complement an earlier study by Sheets *et al*. (2004), which had produced equivocal results with the M-maze test. Like the previous study, it was performed in accordance with EPA guideline OPPTS 870.6300, but with a restricted number of parameters being observed and reported. In comparison with the earlier study, one type of measurement procedure for memory effects was added: the Cincinnati water maze. Whereas the M-maze focuses on associative learning and memory, primarily involving the hippocampus, the labyrinth Cincinnati Water maze does not allow spatial navigation and is a test of path integration ability, requiring an intact entorhinal cortex where information from the hippocampus is integrated.

In short, the study was executed as follows:

Animals were exposed as described above. On post-natal day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 18-20 litters per level, were subjected to evaluation using the following observations and measurements: detailed clinical observations, body weight, tests of spatial learning and memory (i.e., M- and Cincinnati Water Mazes) beginning on PND 60 (± 2 or 4 days, respectively) and an ophthalmic examination. Whole-brain tissue was collected from 10/sex/dietary level (representing 20 litters) on PND 21 and at study termination (approximately 75 days of age) for morphometry.

The following modifications of the guideline were implemented:

- Increased number of offspring for morphometric and neuropathological evaluation (10/sex/dose instead of 6)
- Extended exposure (GD 0 to PND21 instead of GD 6 to PND 10).
- Animals were perfused before collecting brains at the end of exposure on PND 21 and 75.

The first two changes increased the sensitivity of the study, while the last increased the quality of the samples. Therefore, these changes with respect to the guidelines are

considered acceptable.

RESULTS

The achieved intakes of spirodiclofen in this study were lower than those in the earlier study by Sheets *et al*. (2004): 18, 9, and 13% less at low, mid and high dose, respectively, during gestation and 7, 6 and 4% less at low, mid and high dose, respectively, during lactation. As the disputed maze findings of Sheets *et al*. were observed at all dose levels, the lower intakes in this study do not hamper a good re-evaluation of these effects.

Performance in the M-maze test was evaluated on the basis of the number of trials to criterion and the number of errors to criterion. Using these criteria, no treatment related differences were observed between treated and control groups.

An overview of the results is presented in Tables 1 to 4.

TABLE 1 OVERVIEW OF RESULTS FOR DAMS AND LITTER DATA

Dose (mg/kg food)	0	70	350	1500	dr
F-0 dams					
Mortality	0/30	0/30	0/30	0/30	
Clinical signs	no treatment related findings				
Pregnant animals	28	27	29	30	
Body weight					
GD 0-20	no treatment related findings				
LD 0-21	no treatment related findings				
Food consumption					
GD 0-20	no treatment related findings				
LD 0-7				d (5%)	
LD 7-14				d (4%)	
LD 14-21				d (5%)	
FOB	not included in assessment				
Organ weight	not included in assessment				
Pathology					
macroscopy	not included in assessment				
Litter data					
Life foetuses	no treatment related findings				
Viability index	no treatment related findings				
Lactation index	no treatment related findings				
Pup weight					
PND 0-11	no treatment related findings				
PND 17				d (4%)	
PND 21				dc (8%)	
Pup weight gain				dc (9%)	

dr dose related
dc statistically significantly decreased compared to the controls
d decreased, but not statistically significantly compared to the controls

TABLE 2 OVERVIEW OF RESULTS FOR F-1

Dose (mg/kg food)	0		70		350		1500		dr
	m	f	m	f	m	f	m	f	
F-1 animals									
Clinical signs	no treatment related findings								
Body weight (post-weaning)	no treatment related findings								
Food consumption	no treatment related findings								
Sexual maturation	not included in assessment								
Pupil constriction (PND 21)	not included in assessment								
Ophthalmoscopy	no treatment related findings								
FOB	not included in assessment								

Dose (mg/kg food)	0 m	0 f	70 m	70 f	350 m	350 f	1500 m	1500 f	dr
Motor and locomotor activities	not included in assessment								
Acoustic startle	not included in assessment								
Passive avoidance	not included in assessment								
Water mazes									
M-maze									
Learning phase	no treatment related findings								
Retention phase	no treatment related findings								
Cincinnati maze									
Learning phase	no treatment related findings								
Retention phase	no treatment related findings								
Clinical chemistry	not included in assessment								
Brain weight	no treatment related findings								
Pathology									
macroscopy (only brain examined)	no treatment related findings								
microscopic measurements	no treatment related findings								
histopathology	not included in assessment								

dr dose related

TABLE 3 SUMMARY OF RESULTS M-MAZE TEST (MEAN ± STANDARD DEVIATION)

(errors are presented as numbers)

Session	Parameter	Dose (mg/kg food)			
		0	**70**	**350**	**1500**
Males					
Learning Phase	Number of animals	16	16	16	15
	Trials to criterion	6.4±1.4	6.3±2.5	7.9±2.4	7.6±2.1
	Trial 1 - Errors	0.6±0.6	0.6±0.9	0.6±0.8	1.2±1.1
	Trial 2- Errors	0.4±0.5	0.3±0.4	0.7±0.8	1.0±1.0
Retention Phase	Number of animals	16	15	16	15
	Trials to criterion	5.4±1.0	6.5±2.4	5.6±1.4	6.7±2.6
	Trial 1 - Errors	0.2±0.4	0.3±0.5	0.3±0.7	0.1±0.3
	Trial 2 - Errors	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Females					
Learning Phase	Number of animals	16	16	15	16
	Trials to criterion	7.6±2.6	7.3±2.1	8.1±3.1	7.3±2.9
	Trial 1 - Errors	0.8±1.0	0.8±1.1	0.9±1.2	0.4±0.8
	Trial 2 - Errors	0.7±1.1	0.9±1.1	0.7±1.3	0.5±1.0
Retention Phase	Number of animals	15	16	14	15
	Trials to criterion	7.9±3.6	8.4±3.9	8.0±3.8	8.9±4.2
	Trial 1 - Errors	0.0±0.0	0.4±0.9	0.4±0.6	0.1±0.4
	Trial 2 - Errors	0.2±0.6	0.3±0.6	0.1±0.5	0.1±0.4

TABLE 4 SUMMARY OF RESULTS CINCINNATI MAZE TEST (MEAN ± STANDARD DEVIATION)

(latency in seconds; errors are presented as numbers)

Session	Parameter	Dose (mg/kg food)							
		0		70		350		1500	
Males									
Learning Phase	Number of animals	10		10		10		10	
		Latency	**Errors**	**Latency**	**Errors**	**Latency**	**Errors**	**Latency**	**Errors**
	Day 1	300±0	18.5±3.1	286±35	17.3±3.8	300±0	17.8±4.2	289±28	16.6±3.9
	Day 2	241±84	17.6±7.8	227±94	15.8±5.8	217±85	16.0±6.4	269±31	16.7±3.9
	Day 3	175±117	9.9±7.6	118±109	6.0±5.1	104±60	6.5±4.9	171±100	9.7±5.7
	Day 4	80±60	4.8±5.5	100±105	4.8±7.2	48±19	1.8±1.8	96±96	4.1±5.6
	Day 5	52±37	2.0±3.3	63±86	2.1±4.2	48±37	0.9±1.5	44±25	1.1±1.1
Retention Phase	Number of animals	10		9		10		10	
	Day 12	42±19	1.0±1.1	40±26	1.9±3.3	40±16	0.4±0.5	35±21	0.5±0.9
Females									
Learning Phase	Number of animals	10		10		10		10	
		Latency	**Errors**	**Latency**	**Errors**	**Latency**	**Errors**	**Latency**	**Errors**
	Day 1	278±68	20.2±6.4	280±34	22.3±4.0	296±14	21.7±5.9	282±57	21.5±5.8
	Day 2	242±82	17.9±6.0	218±83	16.7±6.3	224±85	17.5±8.2	178±102	16.1±9.4
	Day 3	154±99	11.2±7.6	112±73	8.0±7.0	110±108	5.8±6.1	85±59	6.1±5.3
	Day 4	81±66	4.5±5.5	42±18	2.0±1.4	66±86	2.8±4.7	52±45	2.3±2.8
	Day 5	41±30	1.4±1.9	24±6.3	0.5±0.7	57±87	1.4±2.0	35±19	1.7±1.2
Retention Phase	Number of animals	10		10		9		10	
	Day 12	72±69	4.9±7.4	57±50	1.8±2.9	49±46	2.2±3.6	89±85	5.0±5.8

ACCEPTABLE

The study is considered acceptable as a supplementary study to the study performed by Sheets *et al*. (2004).

Conclusions

Both the M-maze and the Cincinnati maze test demonstrated no significant differences between control and treated groups at any of the dose levels investigated. This confirms the interpretation of the equivocal results of the M-maze test reported by Sheets *et al*. (2004) that there is no effect of spirodiclofen on the performance of the offspring of treated rats in this type of learning and memory test.

Only in the high dose group (1500 mg/kg food), minor effects were observed both in the dams and in the offspring. In the dams these effects were limited to decreases of ca. 5% in food consumption during the lactation period, which were not statically significant and did not lead to decreased body weight and are therefore not considered toxicologically relevant. In the offspring, pup weight (gain) was reduced by ca. 8 to 9% (statistically significant). Based on these effects, the NOAEL for maternal and developmental effects in this study is considered to be respectively 1500 mg/kg food

(equal to 119 mg/kg bw/day) and 350 mg/kg food (equal to 29 mg/kg bw/d). The changes in pup weight were reversible, as after weaning no significant differences in body weight were observed between treated and control animals in the F-1 generation. No neurodevelopmental effects were observed in this study. The effects observed in this study are identical to those observed in the earlier study by Sheets *et al*. (2004), and occurred at the same food level of spirodiclofen.

B.6.12 DERMAL ABSORPTION (ANNEX IIIA 7.3)

In the DAR, the proposed dermal absorption value was 2% for both the concentrate and dilution of spirodiclofen formulated as a 240 SC formulation, based on an *in vivo* study in male monkeys. This *in vivo* study in monkeys was presented in more detail in the revised addendum B6 B7 from September 2006 and was discussed in the expert meeting (EPCO 28, d.d. 27 June – 1 July 2005).

The experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. A number of experts indicated that they would not have accepted the study initially, particularly as there were clear OECD guidelines on both *in vivo* and *in vitro* assessment of dermal absorption. The experts discussed the proposed dermal absorption value of 2%. Areas of concern with the monkey study included the fact that levels of radioactivity in the skin and body were not determined, and the level of total radioactivity recovered (92%). The low level of variation in individual animals supported the theory that the 8% of "lost" radioactivity may have been absorbed, and thus the experts concluded that this should be incorporated into the dermal absorption to give a value of 10%.

Experts considered the physical chemical properties of spirodiclofen, and considered that the molecular weight and K_{OW} supported a dermal absorption value of 10%. It was therefore concluded that the dermal absorption be set at a value of 10% for the concentrate, based on physicochemical properties and supported by the studies in monkeys.

It was additionally noted that no data was available on the dermal absorption potential of the formulation dilution. Therefore a value of 65% was proposed, based on the oral absorption value.

In the revised addendum submitted in September 2006 (not peer reviewed), the RMS re-assessed the EPCO meeting outcomes considering that these conclusions were drawn on wrong assumptions. In summary, the RMS claims that the concentration as tested in the *in vivo* study is an acceptable area dose to be used for the spray dilution. Hence the RMS proposed a dermal absorption value of 10% for the concentrate and spray dilution.

However, the applicant was still of the opinion that a dermal absorption for concentrate and spray dilution of ca 2% is justified, based on the results of an exploratory study in monkeys. According to the RMS, this exploratory study turns out to be a rather important study, however, it was not submitted in the EU-dossier, hence the study itself could not be evaluated. The short summary provided in the conclusion of the main study, and some more details that the applicant made available to the RMS after the EPCO meeting (not peer-reviewed), indeed indicate that a dermal absorption value of ca 2% might be more realistic.

Recently (January 2009), the notifier submitted new dermal absorption data, including the exploratory study in monkeys and a new *in vitro* study. In summary, the notifier conducted three studies which are relevant for the determination of the dermal absorption values for spirodiclofen:

- *in vivo* monkey study (evaluated in the DAR; re-evaluated in the revised addendum from September 2006)
- exploratory *in vivo* monkey study (evaluated in this addendum)
- *in vitro* study with rat and human skin (evaluated in this addendum)

To facilitate the discussion in the expert meeting, all relevant dermal absorption data will be presented in this addendum, including a copy of the already evaluated *in vivo* monkey study by Wu, 2002 (copied from the revised addendum from September 2006).

STUDY 1

Characteristics

Reference	:	Z. Wu (2002)	exposure	:	single dose (occlusion)
type of study	:	*In vivo* dermal absorption	doses	:	151 µg/animal; ca. 6.3 µg/cm^2
year of execution	:	2002	vehicle	:	BAJ2740 SC 240 Blank suspension in water
test substance	:	[Dihydrofuranone-3-^{14}C]BAJ2740 (spirodiclofen) (radiochemical purity 99%)	GLP statement	:	yes
Route	:	dermal	guideline	:	US-EPA 870.7600
Species	:	Rhesus Monkey	acceptability	:	Acceptable
group size	:	5 males			

Study design

The sponsor provided a ready-to-use formulation BAJ2740 SC 240 containing radiolabeled ^{14}C BAJ 2740 (1.51 µg/µL).

Five naïve male rhesus monkeys received a dermal application, under occlusion, of 100 µL of the test substance, containing in total 151 µg 14C-BAJ2740, to the shaven skin (4 cm x 6 cm). Subsequently, the animals were restrained in a primate chair for 8 h and then placed in metabolism cages. At 8 h after dosing the patch was removed, the application site was washed with cotton swabs dipped in soapy water. Next the application site was tape-stripped 16 times, and wiped with isopropyl alcohol swabs and soapy water swabs. Urine, faeces and samples of cage rinse and the final cage wash were collected up to 144 h post dosing. Following removal from the primate chair samples of chair wash were collected. All samples were analyzed for radioactive content.

Results

At 8 h after application 84.53 % of radioactivity was recovered in the dermal washes, with 58.75% being recovered in the first 4 cotton swabs. In tape strips and isopropylalcohol swabs 0.11 and 1.46 % were recovered respectively. From the securing materials and application site patch 2.19 and 1.93 % of radioactivity were recovered respectively. In urine, faeces, cage rinse/wash and chair wash a total of 2.12% (range 1.31 – 3.48%) of administered radioactivity was recovered over 144 h, with about 1.7% being excreted within the first 24 h. Total recovery of radioactivity was 92.34 %.

Table 6.12.1 Total recoveries of BAJ 2740-dihydrofuranone-3-[^{14}C]-derived radioactivity at 144 hours following dermal administration of BAJ 2740 SC 240 containing ^{14}C BAJ 2740 to male Rhesus monkeys at a target dose of 151 µg/animal

Recovery	Sample	Percentage of dose (%)						
		Animal number					Mean	SD
		1001	1002	1003	1004	1005		
Elimination	Urine	1.56	1.98	1.21	1.92	0.50		
	Faeces	0.00	0.07	0.06	0.29	0.08		
	Cage debris/rinse	0.08	1.33	0.00	0.24	0.79		
	Chair/urine pan wash/wipe	0.12	0.1	0.04	0.24	0.00		
	Cage wash/wipe	0.00	0.00	0.00	0.00	0.00		
	Subtotal	**1.76**	**3.48**	**1.31**	**2.69**	**1.37**	**2.12**	**0.94**
Residual	Patch/securing material	4.89	5.96	4.14	4.52	1.08		
	Swabs	81.43	80.72	89.61	84.98	93.21		
	Tape strips	0.08	0.15	0.04	0.14	0.13		
	Subtotal	**86.40**	**86.83**	**93.79**	**89.64**	**94.42**	**90.22**	**3.77**
Total		**88.16**	**90.13**	**95.10**	**92.33**	**95.79**	**92.34**	**3.21**

In some animals elimination still occurred in the last study period (120-144 hours after application): 0.02% in urine and 0.02% in faeces in one animal, 0.14% in faeces in one animal, and 0.10% in cage debris/rinse in one animal.

Table 6.12.2 Elimination of BAJ 2740-dihydrofuranone-3-[^{14}C]-derived radioactivity by male Rhesus monkeys following dermal administration of BAJ 2740 SC 240 containing ^{14}C BAJ 2740 at a target dose of 151 µg/animal

Sample	Time (hours)	Percentage of dose (%)						
		Animal number					Mean	SD
		1001	1002	1003	1004	1005		
Urine	0-4	0.15	0.19	0.00	0.19	0.02		
	4-8	0.60	NS	0.29	0.81	NS		
	8-12	0.55	1.16	0.52	0.46	0.00		
	12-24	0.26	0.30	0.30	0.25	0.34		
	24-48	0.00	0.15	0.10	0.11	0.14		

	48-72	0.00	0.08	0.00	0.08	0.00		
	72-96	0.00	0.02	0.00	0.00	0.00		
	96-120	0.00	0.06	0.00	0.02	0.00		
	120-144	0.00	0.02	0.00	0.00	0.00		
	Subtotal	**1.56**	**1.98**	**1.21**	**1.92**	**0.50**	**1.43**	**0.61**
Faeces	0-4	NS	NS	NS	NS	NS		
	4-8	0.00	0.00	NS	0.00	NS		
	8-12	0.00	NS	NS	NS	NS		
	12-24	0.00	0.00	0.02	0.03	0.00		
	24-48	0.00	0.00	0.04	0.10	0.07		
	48-72	0.00	0.05	0.00	0.00	0.01		
	72-96	0.00	0.00	0.00	0.00	0.00		
	96-120	0.00	0.00	0.00	0.02	0.00		
	120-144	0.00	0.02	0.00	0.14	0.00		
	Subtotal	**0.00**	**0.07**	**0.06**	**0.29**	**0.08**	**0.10**	**0.11**
Cage debris/ rinse	8-12	0.00	0.22	0.00	0.24	0.69		
	12-24	0.00	0.32	0.00	0.00	0.00		
	24-48	0.08	0.00	0.00	0.00	0.00		
	48-72	0.00	0.00	0.00	0.00	0.00		
	72-96	0.00	0.00	0.00	0.00	0.00		
	96-120	0.00	0.79	0.00	0.00	0.00		
	120-144	0.00	0.00	0.00	0.00	0.10		
	Subtotal	**0.08**	**1.33**	**0.00**	**0.24**	**0.79**	**0.49**	**0.56**

Acceptability

In the EPCO meeting, experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. A number of member experts indicated that they would not have accepted the study initially, particularly as there were clear OECD guidelines on both *in vivo* and *in vitro* assessment of dermal absorption. Areas of concern with the monkey study included the fact that levels of radioactivity in the skin and body were not determined, and the level of total radioactivity recovered (92%). The low level of variation in individual animals supported the theory that the 8% of radioactivity lost may have been absorbed, and thus the experts concluded that this should be incorporated into the dermal absorption to give a value of 10%.

Conclusion

It was concluded during the EPCO expert meeting that the *in vivo* dermal absorption of BAJ2710 in rhesus monkeys is approximately 2 + 8 = 10%.

STUDY 2

Characteristics

Reference	:	C. Sebesta (2002)	exposure	:	single dose (occlusion)
type of study	:	*In vivo* dermal absorption	doses	:	ca. 20 µg/cm^2 for the dermal application
year of execution	:	2001	vehicle	:	BAJ2740 SC 240 Blank suspension in water
test substance	:	[Dihydrofuranone-3-^{14}C]BAJ2740 (spirodiclofen) (radiochemical purity 99%)	GLP statement	:	yes
Route	:	Dermal and intravenous	guideline	:	-
Species	:	Rhesus Monkey	acceptability	:	Acceptable as exploratory study
group size	:	1 male			

Study design

The purpose of this study was to determine the rate and route of elimination of BAJ2740-dihydrofuranone-3-^{14}C derived radioactivity following a single intravenous or dermal administration to male rhesus monkeys. This exploratory study was to aid in the design of a definitive dermal absorption and mass balance study.

The study consisted of two groups of one male rhesus monkey per group. BAJ 2740-dihydrofuranone-3- ^{14}C ([^{14}C]-spirodiclofen) was intravenously administered at a target dose of 50 µCi to the animal in Group 1. For the Group 2 animal, an equivalent dermal dose (50 µCi or ca. 20 µg/cm^2) of BAJ 2740 SC 240, containing [^{14}C]-spirodiclofen, was applied, under occlusion, to the shaven skin (4 cm x 6 cm). Subsequently, the animals were restrained in a primate chair for 8 h and then placed in metabolism cages. At 8 h after dermal administration the patch was removed, the application site was washed with 16 cotton swabs dipped in soapy water, 4 isopropyl alcohol swabs and 4 additional cotton swabs dipped in soapy water. At 24 hours and at 48 hours post-dose the application site for the Group 2 animal was tape-stripped 16 times, and and alcohol washes were collected at 48, 168 and 192 hours, and a dermal wipe at 192 hours.

Urine and feces were collected at specified time intervals up to 240 hours post-dose for Group 1 and up to 192 hours post-dose for Group 2. Following the removal of the animal from the primate chair, a chair/urine pan wash/wipe was conducted at specified intervals up to 8 hours post-dose. A cage debris/ cage rinse sample was conducted after each fecal collection up to 240 hours post-dose for Group 1 and up to 192 hours post-dose for Group 2. A cage wash/cage wipe was conducted following the final timepoint. All samples were analyzed for radioactive content by LSC.

Results

Intravenous administration of [^{14}C]-spirodiclofen to a male rhesus monkey resulted in excretion of radioactivity primarily in urine. Total recoveries through 240 hours post-dose were 87.12% in urine, 4.66% in feces, and 15.05% in cage debris/rinse samples. The cage debris/rinse radioactivity can be attributed primarily to urinary excretion based on the fact that the majority of the cage debris/rinse radioactivity was recovered during the first 12 hours after dosing, during which time the animal excreted no fecal matter. Excretion was rapid, with >70% of the dose recovered within 8 hours of dosing and approximately 95% by 24 hours post-dose.

Following dermal application of [^{14}C]-spirodiclofen formulated as 240 SC total recoveries of radioactivity were 1.11% in urine, 0.25% in feces (of which 0.22% was from the fecal-contaminated glove sample), and 0.34% in cage debris/rinse samples through 192 hours post-dose, suggesting minimal systemic exposure to [^{14}C]-spirodiclofen-derived radioactivity. The majority of the excreted radioactivity was recovered within 24 hours of dosing.

The overall recovery of radioactivity for the intravenously dosed animal was 107.27%. The overall recovery of radioactivity for the dermally dosed animal was 108.30%, with the large majority associated with the residual radioactivity recovered from the application site. The dermal absorption was estimated to be 1.77% of the administered dose.

Table 6.12.3 Total recoveries of [^{14}C]-spirodiclofen-derived radioactivity at 240 hours following intravenous administration and 192 hours following dermal administration to male Rhesus monkeys at a target dose of 50 µCi

Recovery	Sample	Percentage of dose (%)	
		Intravenous (Group 1)	Dermal (Group 2)
Elimination	Urine	87.12	1.11
	Faeces	4.66	0.25 [a]
	Cage debris/rinse	15.05	0.34
	Chair/urine pan wash/wipe	NA	0.07
	Cage wash	0.20	0.00
	Cage wipe	0.24	0.00
	Subtotal	**107.27**	**1.77**
Residual	Patch/securing material	NA	8.67
	Swabs	NA	97.78
	Tape strips	NA	0.08
	Subtotal	**NA**	**106.53**
Total		**107.27**	**108.30**

NA Not applicable
a residual radioactivity from the glove sample (0.22%) included in the data

After intravenous administration, elimination still occurred in the last study period (216-240 hours after dosing): 0.03% in urine. After dermal application there were serial nondetects starting 72 hours post-dose.

Table 6.12.4 Elimination of [^{14}C]-spirodiclofen-derived radioactivity by male Rhesus Monkeys following intravenous administration of [^{14}C]-spirodiclofen and dermal administration of BAJ 2740 SC 240 containing [^{14}C]-spirodiclofen at a target dose of 50 µCi

Recovery	Sample	Percentage of dose (%)	
		Intravenous (Group 1)	Dermal (Group 2)
Urine	0-4	39.85	0.03
	4-8	24.50	0.53
	8-12	10.13	0.00
	12-24	8.01	0.30
	24-48	3.49	0.18
	48-72	0.41	0.07
	72-96	0.14	0.00
	96-120	0.16	0.00
	120-144	0.10	0.00
	144-168	0.17	0.00
	168-192	0.10	0.00
	192-216	0.03	NA
	216-240	0.03	NA
	Subtotal	**87.12**	**1.11**

Faeces	0-4	NS	NS
	4-8	NS	0.22 [a]
	8-12	NS	NS
	12-24	0.10	0.00
	24-48	2.88	0.02
	48-72	1.48	0.00
	72-96	0.20	0.00
	96-120	0.00	0.00
	120-144	0.00	0.00
	144-168	0.00	0.00
	168-192	0.00	0.01
	192-216	0.00	NA
	216-240	0.00	NA
	Subtotal	**4.66**	**0.25**
Cage debris/ rinse	0-4	5.23	NS
	4-8	4.10	NS
	8-12	2.09	0.34
	12-24	1.12	0.00
	24-48	0.90	0.00
	48-72	0.41	0.00
	72-96	0.50	0.00
	96-120	0.39	0.00
	120-144	0.16	0.00
	144-168	0.00	0.00
	168-192	0.00	0.00
	192-216	0.15	NA
	216-240	0.00	NA
	Subtotal	**15.05**	**0.34**

NS No sample excreted
NA Not applicable
a residual radioactivity from the glove sample (0.22%) included in the data

Acceptability

The study is acceptable as exploratory study.

Conclusion

Based on the results obtained in one animal, the *in vivo* dermal absorption of spirodiclofen in rhesus monkeys is approximately 2% (rounded value).

STUDY 3

Characteristics

reference	:	Odin-Feurtet, M. (2008)	exposure	:	8 h, unoccluded
type of study	:	*in vitro* dermal absorption	doses	:	2.4 mg/cm^2 (concentrate), 16 µg/cm^2 (intermediate dose) and 0.5 µg/cm^2 (low dose)
year of execution	:	2008	vehicle	:	Blank formulation Envidor SC 240
test substance	:	Radiolabelled test substance: [Dihydrofuranone-3-^{14}C]-spirodiclofen, radiochemical purity 99%, specific activity 3.89 MBq/mg. Unlabelled test substance: spirodiclofen, purity 99.2%.	GLP statement	:	yes
route	:	Dermal	guideline	:	OECD guideline 428
species	:	Human and rat	acceptability	:	acceptable
group size	:	4, 5 or 6 replicates (see Table 6.12.5 for details per group)	**Result**	:	Human skin: 0.33% (concentrate; 2.4 mg/cm^2), 1.66% (at 16 µg/cm^2) and 3.1% (at 0.5 µg/cm^2) Rat skin: 8.2% (concentrate; 2.4 mg/cm^2), 24.4% (at 16 µg/cm^2) and 15.7% (at 0.5 µg/cm^2)

Study design

The percutaneous absorption of [^{14}C]-triflumizole was studied *in vitro*, using flow-through diffusion cells. Dermatomed skin from rats and humans was exposed to either the neat product (240 g spirodiclofen/L formulation resulting in an area dose of 2.4 mg/cm^2), or two spray dilutions (1.6 g spirodiclofen/L formulation resulting in an area dose of 16 µg/cm^2 and 0.05 g spirodiclofen/L formulation resulting in an area dose of 0.5 µg/cm^2). The integrity of the skin membranes was established prior to the application of the test substance, by measuring the trans-epidermal water loss (TEWL). Aliquots of 10 µl were applied to an area of 1 cm^2 of unoccluded skin samples. The test substance remained in contact with the skin for 8 hours. The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumine and gentamycin. Samples of receptor fluid were collected hourly for 24 hours. At the end of the exposure period, the test compound was removed from the skin surface with 1% v/v Tween 80 in PBS using natural sponge swabs. At the end of the study (24 hours after application) the skin samples were swabbed again and each skin sample was tape stripped to remove the *stratum corneum*. The tape strips were individually collected and analysed. The receptor fluid, skin swabs, tape strips, skin membranes (skin and surrounding skin) and diffusion cell components were analysed using LSC.

Results

The solubility of spirodiclofen in the receptor fluid was sufficient. The dose formulations were considered to be homogeneous and acceptable for use in the study.

Table 6.12.5 presents the distribution of radioactivity for the human and rat dermatomed skin following a single topical application of the high, intermediate and low dose concentration of [^{14}C]-spirodiclofen in Envidor SC 240 formulation.

Table 6.12.5 Recovery of radioactivity for rat and human skin membranes

Absorption is expressed as percentage of the administered radiolabel.

High dose (240 g/L)				
	Human (n = 5)		Rat (n = 5)	
Samples	Mean	SD	Mean	SD
Surface dose (tape strips 1+2)	0.27	0.13	3.54	2.94
Skin swabs [a]	104.81	2.14	93.01	4.66
Donor chamber	0.03	0.05	0.17	0.16
Total % non absorbed	105.44	2.14	96.72	4.05
Skin [b]	0.11	0.11	0.65	0.39
Stratum corneum [c]	0.23	0.20	7.48	3.05
Total % at dose site	0.33	0.23	8.13	3.23
Total % directly absorbed [d]	0.002	0.005	0.085	0.03
Total % potentially absorbable [e]	0.33	0.31	8.22	3.25
Total % Recovery	105.44	2.16	104.93	1.09
Intermediate Dose (1.6 g/L)				
	Human (n = 4)		Rat (n = 4)	
Samples	Mean	SD	Mean	SD
Surface dose (tape strips 1+2)	1.19	0.89	3.94	1.87
Skin swabs [a]	98.33	1.32	72.15	10.15
Donor chamber	0.08	0.07	0.10	0.11
Total % non absorbed	99.60	1.27	76.20	8.37
Skin [b]	0.70	0.63	7.02	5.67
Stratum corneum [c]	0.95	0.60	13.58	8.82
Total % at dose site	1.65	1.22	20.61	8.84
Total % directly absorbed [d]	0.012	0.01	3.82	1.27
Total % potentially absorbable [e]	1.66	1.22	24.42	8.43
Total % Recovery	101.27	2.03	100.62	1.19
Low Dose (0.05 g/L)				
	Human (n = 6)		Rat (n = 4)	
Samples	Mean	SD	Mean	SD
Surface dose (tape strips 1+2)	3.82	2.78	5.56	2.27
Skin swabs [a]	88.64	2.61	74.38	4.19
Donor chamber	0.00	0.00	1.45	1.18
Total % non absorbed	92.46	2.02	81.39	1.18
Skin [b]	1.01	0.53	3.08	2.61
Stratum corneum [c]	2.02	1.54	8.71	3.58
Total % at dose site	3.03	1.92	11.79	4.34
Total % directly absorbed [d]	0.11	0.25	3.91	2.53
Total % potentially absorbable [e]	3.14	1.96	15.70	1.89
Total % Recovery	95.60	3.14	97.09	1.13

[a]: including swabs at 8 and 24 hours + surrounding swabs

[b]: Sum of skin after tape-stripping procedure and surrounding skin

[c]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.

[d]: including receptor fluid (0 to 24h) receptor fluid at termination time and receptor chamber

[e]: total % directly absorbed + total % at dose site

SD: standard deviation

n: number of skin cells used for calculation

Total mean recovery of the high, intermediate and low dose was 105.4, 101.3 and 95.6% for human skin and 104.9, 100.6 and 97.1% for rat skin. Results are given in Table 6.12.5. At the high dose 0.002% AR (applied radioactivity) had penetrated through the human skin at the 24 hour time point. Rat skin exposed under the same conditions was more permeable, as 0.085% AR penetrated within 24 hours. At the intermediate dose 0.012% and 3.82% of the applied dose penetrated within 24 hours through human and rat skin, respectively. At the low dose 0.11% and 3.91% of the applied dose penetrated within 24 hours through human and rat skin, respectively.

Acceptability

The study was performed in accordance with draft OECD 428 and is considered acceptable.

Conclusion

Since the swabbing procedure was intended to reflect a simple washing regimen at the end of the working day, the amount of radioactivity retrieved in this compartment was considered to be nonabsorbed. Since the material recovered in the surface tape-strips (first two tape-strips) could be associated with surface residues following incomplete removal of the dose after an 8-hour exposure period and/or material from the superficial *stratum corneum*, the amount of radioactivity retrieved in this compartment was considered to be non-absorbed (this is in line with the EFSA list of decisions).

Good recovery data were obtained, with mean total recoveries of radioactivity in the range of 95.6% to 105.4% of the applied dose.

For both the neat and diluted formulations, the majority of the radioactivity was removed by swabbing and by removal of the surface dose (first two tape strips).

An overview of the different compartments is presented in Table 6.12.6.

The mean percentage of [^{14}C]-spirodiclofen formulated as Envidor 240 SC considered to be potentially absorbable (directly absorbed plus total remaining at dose site) over a period of 24 hours for the neat formulation was 0.334% and 8.22% for the human and

rat skin, respectively, yielding a factor difference of 25 between the two species for the neat product.

The mean percentage of [^{14}C]-spirodiclofen formulated as Envidor 240 SC to be potentially absorbable for the intermediate dose formulation was 1.66% and 24.4% for the human and rat skin respectively, yielding a factor difference of 15 between the two species for the intermediate dose formulation.

The mean percentage of [^{14}C]-spirodiclofen formulated as Envidor 240 SC to be potentially absorbable for the low dose formulation was 3.14% and 15.7% for the human and rat skin respectively, yielding a factor difference of 5 between the two species for the low dose formulation.

Table 6.12.6 Recovery of radioactivity for rat and human skin membranes

Absorption is expressed as percentage of the administered radiolabel.

	Distribution of radioactivity					
Dose levels	Neat formulation (SYP13262, 240 g/L)		Spray dilution: Intermediate dose (SYP13264, 1.6 g/L)		Spray dilution: Low dose (SYP13265, 0.05 g/L)	
Species	Human (n = 5)	Rat (n = 5)	Human (n = 4)	Rat (n = 4)	Human (n = 6)	Rat (n = 4)
SURFACE COMPARTMENT						
Skin swabs [a]	104.808	93.008	98.335	72.154	88.643	74.379
Surface Dose (tape-strips1&2)	0.266	3.536	1.187	3.938	3.817	5.561
Donor chamber	0.032	0.173	0.081	0.105	ND	1.446
Total % non-absorbed	***105.106***	***96.717***	***99.603***	***76.197***	***92.459***	***81.385***
SKIN COMPARTMENT						
Stratum corneum [b]	0.226	7.478	0.947	13.581	2.024	8.709
Skin [c]	0.106	0.654	0.704	7.024	1.005	3.082
Total % at dose site	***0.332***	***8.132***	***1.651***	***20.605***	***3.029***	***11.791***
RECEPTOR COMPARTMENT						
Total % directly absorbed [d]	***0.002***	***0.085***	***0.012***	***3.816***	***0.112***	***3.910***
TOTAL ABSORBABLE						
Total % potentially absorbable [e]	***0.334***	***8.217***	***1.663***	***24.421***	***3.141***	***15.702***
Total % Recovery	**105.441**	**104.934**	**101.266**	**100.618**	**95.600**	**97.087**

[a]: including swabs at 8 and 24 hours + surrounding swabs

[b]: tape-strips excluding number 1 & 2 which are considered to be non-absorbed dose.

[c]: sum of skin after tape-stripping procedure and surrounding skin

[d]: including receptor fluid (0 to 24 h), receptor fluid at termination time and receptor chamber

[e]: total % directly absorbed + total % at dose site

n: number of skin cells used for calculation

ND: not detected (below the limit of detection)

Overall conclusion on dermal absorption

During the EPCO meeting the *in vivo* dermal absorption study by Wu (2002) was discussed and experts expressed concerns relating to both the ethics of conducting dermal absorption studies on monkeys, and the quality of the data. Hence experts considered the physical chemical properties of spirodiclofen, and considered that the molecular weight and K_{OW} supported a dermal absorption value of 10%. It was therefore concluded that the dermal absorption be set at a value of 10% based on physicochemical properties and supported by the study in monkeys.

It was additionally noted that no data was available on the dermal absorption potential of the formulation dilution. Therefore a value of 65% was proposed, based on the oral absorption value.

However, a re-assessment by the RMS after the EPCO meeting revealed that these conclusions were drawn on wrong assumptions. According to the GAP the concentrated formulation contains 240 g as/L, and the spray dilution is 0.048-0.096 g as/L. In the *in vivo* study a dose of 1.51 g/L was tested, which is a factor of over 150 lower than the concentrate and a factor of 16 to 32 higher than the spray dilution. Moreover, the area dose applied to the monkeys was 151 µg/24 cm^2 = 6.3 µg/cm^2. This rather low area dose is an acceptable area dose to be used for the spray dilution. Furthermore, as the specific activity of the radiolabelled spirodiclofen was 3.9 MBq/mg, the spray dilution concentrations were technically not feasible as they would have resulted in an unacceptable loss of sensitivity (only 19-38 kBq per animal). Hence the conclusion drawn in the EPCO meeting (10% for the concentrate and 65% for the spray dilution) is not justified and a dermal absorption value of 10% for the concentrate and spray dilution should be derived based on the *in vivo* monkey study. This is also more in line with the general practice of applying one default value for both the concentrate and the spray dilution.

However, the exploratory study in monkeys evaluated in this addendum shows that the assumption that 8% of “lost” radioactivity in the study by Wu (2002) may have been absorbed and should be incorporated into the dermal absorption, is wrong. The total recovery of the i.v. part of the exploratory study (107%) indicated that the administered dose was not retained in the body. This is also supported by the data obtained in the rat metabolism studies (see ADME studies in DAR) that demonstrated that overall excretion of orally administered [^{14}C]-spirodiclofen-derived radioactivity was fast and almost complete within 48 hours after administration. Tissue distribution investigations in the rat also demonstrated that spirodiclofen and its metabolites have no tendency to bind irriversibly to or accumulate in any organ or tissue.

The total recovery after dermal application (108%) was comparable to the recovery after i.v. administration and dermal absorption was about 2%, confirming the results (2% dermal absorption) of the study by Wu.

The new *in vitro* dermal absorption study has been performed with spirodiclofen formulated as Envidor SC 240, which is the representative formulation evaluated in the EU. The dose levels of the concentrate (the neat formulation) and the low dose (0.05 g spirodiclofen/L formulation) are representative for the intended uses. Furthermore, human skin has been used in the *in vitro* study. Therefore, the RMS

considers the results of the *in vitro* dermal absorption study most relevant for the risk assessment of Envidor SC 240. The results show that the amount directly absorbed (amount in receptor fluid) is very low. However, the dermal depot should also be taken into account as potentially absorbed. For the concentrate a value of 0.4% (rounded value) will be used in the risk assessment and for the spray dilution a value of 3% (rounded value) will be used. The value for the spray dilution is rounded to 3%, because the amount directly absorbed is only 0.1% and this value is furthermore supported by the results of the *in vivo* monkey study (2% dermal absorption for the spray dilution).

B.6.14 Exposure data (annex iiia 7.2)

In the DAR, the proposed dermal absorption value was 2% for both concentrate and diluted spirodiclofen, based on an *in vivo* study in male monkeys. In the revised addendum B6 B7 from September 2006 (included in the final addendum from November 2006), the exposure data were re-calculated with a dermal absorption value of 10% for the concentrate and spray dilution.

In the addendum from November 2006 (included in the final addendum from November 2006), the exposure data were re-calculated with dermal absorption values of 10% (concentrate) and 65% (dilution), as agreed upon during the Expert meeting, although the RMS already noted that these values were derived based on wrong assumptions. The EFSA conclusion was based on 10 and 65% dermal absorption, resulting in an exposure exceeding the AOEL for the worker and bystander. However, EFSA did note in their conclusion that the exposure would be below the AOEL if 10% dermal absorption would be considered for the concentrate and spray dilution.

Based on the new *in vitro* dermal absorption study, the RMS now proposes a dermal absorption value of 0.4% for the concentrate and 3% for the spray dilution. Therefore, re-calculated exposure data are presented below.

Product information

Product : Envidor SC 240

Purpose: Acaricide/insecticide

Active substance (a.s.): spirodiclofen 240 g a.s./L

	Suspension Concentrate (SC)
Package size:	1 L bottle (42.0 mm aperture), 5 L can (54.7 mm aperture) and 10 L can (54.7 mm aperture), HDPE

Internal operator exposure values without and with personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM).

For bystander exposure during manual or mechanical spraying outdoors, no formally approved models exist. As an estimate, the draft values proposed for the EUROPOEM II, 2002 model were used. These values represent the 90th percentile exposure values for bystanders. Since bystanding should as much as possible be prevented and will usually occur incidentally, it cannot be assumed that bystanders will be using any kind of personal protective equipment, therefore the use of this equipment is not considered in bystander risk assessment.

For worker exposure during re-entry activities, the values proposed for the EUROPOEM II, 2002 model were used.

The semi-chronic/chronic AOEL of 0.009 mg/kg bw/d (=0.63 mg for a 70-kg person) is used for the risk assessment (see list of endpoints in the EFSA conclusion). Based on the results from the *in vitro* dermal absorption study, a dermal absorption value of 0.4 and 3% was derived for the concentrate and spray dilution of Envidor SC 240, respectively. For respiratory exposure a default value of 100% was used, *i.e.* internal exposure equals external exposure.
The basic assumptions, input data and calculations used in the risk assessment are further specified below.

B.6.14.1 Operator exposure (IIIA 7.2.1)

Mechanical or manual upward spraying on grape

Application technique	: tractor mounted upwards high volume sprayers or knapsack

Input data

Concentration a.s. in formulation	:	240 g a.s. /L
Spray volume	:	1000 L/ha
Concentration in spray liquid	:	0.0096 g a.s. /hL
Application rate	:	0.096 kg a.s./ha (0.4 L product/ha)

Mechanical or manual upward spraying on pome fruits and stone fruits

Application technique	:	tractor mounted upwards high volume sprayers or knapsack

Input data

Concentration a.s. in formulation	:	240 g a.s. /L
Spray volume	:	1500 L/ha
Concentration in spray liquid	:	0.0096 g a.s. /hL
Application rate	:	0.144 kg a.s./ha (0.6 L product/ha)

Mechanical or manual upward spraying on citrus

Application technique	:	tractor mounted upwards high volume sprayers or knapsack

Input data

Concentration a.s. in formulation	:	240 g a.s. /L
Spray volume	:	3000 L/ha
Concentration in spray liquid	:	0.0048 g a.s. /hL
Application rate	:	0.144 kg a.s./ha (0.6 L product/ha)

B.6.14.1.1 Exposure estimates with UK-POEM

THE MODEL IS LARGELY BASED ON UNPUBLISHED STUDIES, CARRIED OUT IN THE UK BY INDUSTRY AND MAFF. 75TH PERCENTILES ARE CALCULATED.

B.6.14.1.1.1 Mechanical upward spraying on grape without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.4**	l/ha	Work rate/day	**15**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0.06	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.06	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application		
Dermal exposure	0.06	ml/day		121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml		0.096	mg/ml
Dermal exposure to a.s.	14.4	mg/day		11.6352	mg/day
Percent absorbed	0.4	%		3	%
Absorbed dose	0.0576	mg/day		0.349056	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.435456	mg/day
Operator body weight	60	kg
Operator exposure	0.0072576	mg/kg bw/day

B.6.14.1.1.2 Mechanical upward spraying on grape with PPE (gloves during mixing/loading)

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	None	
Dose	**0.4**	l/ha	Work rate/day	**15**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	15	ha/day
Number of operations	6	/day
Hand contamination	0.06	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.006	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application		
Dermal exposure	0.006	ml/day	121.2	ml/day	
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml	
Dermal exposure to a.s.	1.44	mg/day	11.6352	mg/day	
Percent absorbed	0.4	%	3	%	
Absorbed dose	0.00576	mg/day	0.349056	mg/day	

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.383616	mg/day
Operator body weight	60	kg
Operator exposure	0.0063936	mg/kg bw/day

B.6.14.1.1.3 Mechanical upward spraying on pome fruits/stone fruits without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.09	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	21.6	mg/day	11.6352	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.0864	mg/day	0.349056	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.464256	mg/day
Operator body weight	60	kg
Operator exposure	0.0077376	mg/kg bw/day

B.6.14.1.1.4 Mechanical upward spraying on pome fruits/stone fruits with PPE (gloves during mixing/loading)

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Product	**Envidor SC 240**	Active substance	**spirodiclofen**	
Formulation type	organic solvent-based	a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4** %	Dermal absorption from spray	**3**	%
Container	1 litre any closure			
PPE during mix/loading	Gloves	PPE during application	None	
Dose	**0.6** l/ha	Work rate/day	**15**	ha
Application volume	**1500** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	1500	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application		
Dermal exposure	0.009	ml/day	121.2	ml/day	
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml	
Dermal exposure to a.s.	2.16	mg/day	11.6352	mg/day	
Percent absorbed	0.4	%	3	%	
Absorbed dose	0.00864	mg/day	0.349056	mg/day	

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.0288	mg/day
Percent absorbed	100	%
Absorbed dose	0.0288	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.386496	mg/day
Operator body weight	60	kg
Operator exposure	0.0064416	mg/kg bw/day

B.6.14.1.1.5 Mechanical upward spraying on citrus without PPE

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.09	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.09	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	21.6	mg/day	5.8176	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.0864	mg/day	0.174528	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.0144	mg/day
Percent absorbed	100	%
Absorbed dose	0.0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.275328	mg/day
Operator body weight	60	kg
Operator exposure	0.0045888	mg/kg bw/day

B.6.14.1.1.6 Mechanical upward spraying on citrus with PPE (gloves during mixing/loading)

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**15**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	15	ha/day
Number of operations	9	/day
Hand contamination	0.09	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.009	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed broadcast air-assisted sprayer: 500 l/ha			
Application volume	3000	spray/ha		
Volume of surface contamination	400	ml/h		
Distribution	Hands	Trunk	Legs	
	10%	65%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	2%	5%	
Dermal exposure	10	5.2	5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.009	ml/day	121.2	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	2.16	mg/day	5.8176	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.00864	mg/day	0.174528	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.05	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.0144	mg/day
Percent absorbed	100	%
Absorbed dose	0.0144	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.197568	mg/day
Operator body weight	60	kg
Operator exposure	0.0032928	mg/kg bw/day

B.6.14.1.1.7 Manual upward spraying on grapes without PPE

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (400 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target		
Product	**Envidor SC 240**	Active substance	**spirodiclofen**
Formulation type	organic solvent-based	a.s. concentration	**240** mg/ml
Dermal absorption from product	**0.4** %	Dermal absorption from spray	**3** %
Container	1 litre any closure		
PPE during mix/loading	None	PPE during application	None
Dose	**0.4** l/ha	Work rate/day	**0.4** ha
Application volume	**1000** l/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	0.4	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	64.8	mg/day	9.792	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.2592	mg/day	0.29376	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.56448	mg/day
Operator body weight	60	kg
Operator exposure	0.009408	mg/kg bw/day

B.6.14.1.1.8 Manual upward spraying on grapes with PPE (gloves during mixing/loading)

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (400 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	Gloves		PPE during application	None	
Dose	**0.4**	l/ha	Work rate/day	**0.4**	ha
Application volume	**1000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.4	litres product/ha
Work rate	0.4	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	6.48	mg/day	9.792	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.02592	mg/day	0.29376	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.3312	mg/day
Operator body weight	60	kg
Operator exposure	0.00552	mg/kg bw/day

B.6.14.1.1.9 Manual upward spraying on pome fruits/stone fruits without PPE

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (600 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**0.267**	ha
Application volume	**1500**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.266666667	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	64.8	mg/day	9.792	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.2592	mg/day	0.29376	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.56448	mg/day
Operator body weight	60	kg
Operator exposure	0.009408	mg/kg bw/day

B.6.14.1.1.10 Manual upward spraying on pome fruits/stone fruits with PPE (gloves during mixing/loading)

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (600 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Product	**Envidor SC 240**		Active substance	**spirodiclofen**
Formulation type	organic solvent-based		a.s. concentration	**240** mg/ml
Dermal absorption from product	**0.4** %		Dermal absorption from spray	**3** %
Container	1 litre any closure			
PPE during mix/loading	Gloves		PPE during application	None
Dose	**0.6** l/ha		Work rate/day	**0.267** ha
Application volume	**1500** l/ha		Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.266666667	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	1500	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.096	mg/ml
Dermal exposure to a.s.	6.48	mg/day	9.792	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.02592	mg/day	0.29376	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.096	mg/ml
Inhalation exposure to a.s.	0.01152	mg/day
Percent absorbed	100	%
Absorbed dose	0.01152	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.3312	mg/day
Operator body weight	60	kg
Operator exposure	0.00552	mg/kg bw/day

B.6.14.1.1.11 Manual upward spraying on citrus without PPE

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (1200 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target				
Product	**Envidor SC 240**		Active substance	**spirodiclofen**	
Formulation type	organic solvent-based		a.s. concentration	**240**	mg/ml
Dermal absorption from product	**0.4**	%	Dermal absorption from spray	**3**	%
Container	1 litre any closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**0.6**	l/ha	Work rate/day	**0.133**	ha
Application volume	**3000**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.133333333	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.27	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.27	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	64.8	mg/day	4.896	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.2592	mg/day	0.14688	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.00576	mg/day
Percent absorbed	100	%
Absorbed dose	0.00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.41184	mg/day
Operator body weight	60	kg
Operator exposure	0.006864	mg/kg bw/day

B.6.14.1.1.12 Manual upward spraying on citrus with PPE (gloves during mixing/loading)

(FOR MANUAL SPRAYING THE MODULE USING THE 15 L TANK HAS BEEN CHOOSEN, BECAUSE THE MODULE FOR OUTDOOR HIGH LEVEL TARGET WITH A 2.5 L TANK IS NOT REALISTIC (1200 OPERATIONS/DAY FOR MIXING AND LOADING).

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Product	**Envidor SC 240**		Active substance	**spirodiclofen**
Formulation type	organic solvent-based		a.s. concentration	**240** mg/ml
Dermal absorption from product	**0.4** %		Dermal absorption from spray	**3** %
Container	1 litre any closure			
PPE during mix/loading	Gloves		PPE during application	None
Dose	**0.6** l/ha		Work rate/day	**0.133** ha
Application volume	**3000** l/ha		Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	1	litres
Hand contamination/operation	0.01	ml
Application dose	0.6	litres product/ha
Work rate	0.133333333	ha/day
Number of operations	27	/day
Hand contamination	0.27	ml/day
Protective clothing	Gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.027	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Hand-held sprayer (15 l tank): hydraulic nozzles. Outdoor, low level target			
Application volume	3000	spray/ha		
Volume of surface contamination	50	ml/h		
Distribution	Hands	Trunk	Legs	
	25%	25%	50%	
Clothing	None	Permeable	Permeable	
Penetration	100%	20%	18%	
Dermal exposure	10	2.5	4.5	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	102	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.027	ml/day	102	ml/day
Concen. of a.s. product or spray	240	mg/ml	0.048	mg/ml
Dermal exposure to a.s.	6.48	mg/day	4.896	mg/day
Percent absorbed	0.4	%	3	%
Absorbed dose	0.02592	mg/day	0.14688	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.02	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.048	mg/ml
Inhalation exposure to a.s.	0.00576	mg/day
Percent absorbed	100	%
Absorbed dose	0.00576	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.17856	mg/day
Operator body weight	60	kg
Operator exposure	0.002976	mg/kg bw/day

B.6.14.1.2 Exposure estimates with the German model

The German model is based on unpublished studies performed by industry and all carried out in Germany. The chosen statistic is the geometric mean (GM).

B.6.14.1.2.1 Mechanical upward spraying in grapes without and with PPE (gloves during mixing/loading)

= HIGH_CROP_TRACTOR_MOUNTED =

	Treated area per day	**A =**	**8**	**ha/d**	at BBA = 8
	Use rate	**R =**	***0.096***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0.0006	0.07	0.008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0.0006***	0.096	8	0.0004608	mg/pers. x d
Ia = I*a x R x A	0.018	0.096	8	0.013824	mg/pers. x d
		I, in total =		**0.0142848**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.096	8	1.8432	mg/pers. x d
Da/h = D*a/h x R x A	0.7	0.096	8	0.5376	mg/pers. x d
Da/c = D*a/c x R x A	1.2	0.096	8	0.9216	mg/pers. x d
Da/b = D*a/b x R x A	9.6	0.096	8	7.3728	mg/pers. x d
		D, in total =		**10.6752**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.0004608	-	1		0.0004608 mg/pers. x d
Ia =	0.013824	-	1		0.013824 mg/pers. x d
					0.0142848 mg/pers. x d

Estimated derm. exp.

Dm/h =	1.8432	SS 110	0.01		0.018432 mg/pers. x d
Da/h =	0.5376		1		0.5376 mg/pers. x d
Da/c =	0.9216		1		0.9216 mg/pers. x d
Da/b =	7.3728		1		7.3728 mg/pers. x d
					8.850432 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0004608	0.0004608	0.0004608	0.0004608
Inhalation: appl.	100%	0.013824	0.013824	0.013824	0.013824
Dermal: m/l	0.4%	1.8432	0.018432	0.0073728	7.3728E-05
Dermal: appl.	3%	8.832	8.832	0.26496	0.26496
			mg/pers./d:	0.2866176	0.27931853
kg bw:	70		mg/kg bw/d:	0.00409454	0.00399026

syst. AOEL:	0.009	**% of AOEL:**	**45.4948571**	**44.3362743**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

B.6.14.1.2.2 Mechanical upward spraying in pome fruits, stone fruits and citrus without and with PPE (gloves during mixing/loading)

= HIGH_CROP_TRACTOR_MOUNTED =

	Treated area per day	**A =**	**8**	**ha/d**	at BBA = 8
	Use rate	**R =**	***0.144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	**solid: WG**	I*a = 0,018	D*a/c = 1,2
I*m	0.0006	0.07	0.008	D*a/h = 0,7	D*a/b = 9,6
D*m/h	2.4	6	2		

Estimated inhalation exposure:

Im = I*m x R x A	***0.0006***	0.144	8	0.0006912	mg/pers. x d
Ia = I*a x R x A	0.018	0.144	8	0.020736	mg/pers. x d
		I, in total =		**0.0214272**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***2.4***	0.144	8	2.7648	mg/pers. x d
Da/h = D*a/h x R x A	0.7	0.144	8	0.8064	mg/pers. x d
Da/c = D*a/c x R x A	1.2	0.144	8	1.3824	mg/pers. x d
Da/b = D*a/b x R x A	9.6	0.144	8	11.0592	mg/pers. x d
		D, in total =		**16.0128**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.0006912	-	1		0.0006912 mg/pers. x d
Ia =	0.020736	-	1		0.020736 mg/pers. x d
					0.0214272 mg/pers. x d

Estimated derm. exp.

Dm/h =	2.7648	SS 110	0.01		0.027648 mg/pers. x d
Da/h =	0.8064		1		0.8064 mg/pers. x d
Da/c =	1.3824		1		1.3824 mg/pers. x d
Da/b =	11.0592		1		11.0592 mg/pers. x d
					13.275648 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.0006912	0.0006912	0.0006912	0.0006912
Inhalation: appl.	100%	0.020736	0.020736	0.020736	0.020736
Dermal: m/l	0.4%	2.7648	0.027648	0.0110592	0.00011059
Dermal: appl.	3%	13.248	13.248	0.39744	0.39744
			mg/pers./d:	0.4299264	0.41897779
kg bw:	70		mg/kg bw/d:	0.00614181	0.0059854
syst. AOEL:	0.009		**% of AOEL:**	**68.2422857**	**66.5044114**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**

B.6.14.1.2.3 Manual upward spraying in grapes without and with PPE (gloves during mixing/loading)

For manual spraying with a 15 L knapsack, a treated area of 0.45 ha is used. It is assumed that 6 tanks can be sprayed in one hour, and manual spraying takes 5 hours. So 30 tanks can be sprayed. Taking into account the tank volume of 15 L, the maximum area treated will be 0.45 ha (15 L x 30 operations / 1000 L/ha). This is consistent with the assumed 0.4 ha treated manually in the UK-POEM model.

= HIGH_CROP_HAND_HELD (HCHH) = **(at BBA)**

	Treated area per day	**A =**	***0.45***	**ha/d**	**(1 ha/d)**
	Use rate	**R =**	***0.096***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	I*a = 0,3	D*a/c = 4,8
I*m	0.05	0.8	0.02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = I*m x R x A	***0.05***	0.096	0.45	0.00216	mg/pers. x d
Ia = I*a x R x A	0.3	0.096	0.45	0.01296	mg/pers. x d
			I, in total =	**0.01512**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0.096	0.45	8.856	mg/pers. x d
Da/h = D*a/h x R x A	10.6	0.096	0.45	0.45792	mg/pers. x d
Da/c = D*a/c x R x A	4.8	0.096	0.45	0.20736	mg/pers. x d
Da/b = D*a/b x R x A	25	0.096	0.45	1.08	mg/pers. x d
			D, in total =	**10.60128**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.00216	-	1	0.00216	mg/pers. x d
Ia =	0.01296	-	1	0.01296	mg/pers. x d
				0.01512	**mg/pers. x d**

Estimated derm. exp.

Dm/h =	8.856	SS 110	0.01	0.08856	mg/pers. x d
Da/h =	0.45792		1	0.45792	mg/pers. x d
Da/c =	0.20736		1	0.20736	mg/pers. x d
Da/b =	1.08		1	1.08	mg/pers. x d
				1.83384	**mg/pers. x d**

		Estimated exposure		Systemic exposure	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.00216	0.00216	0.00216	0.00216
Inhalation: appl.	100%	0.01296	0.01296	0.01296	0.01296
Dermal: m/l	0.4%	8.856	0.08856	0.035424	0.00035424
Dermal: appl.	3%	1.74528	1.74528	0.0523584	0.0523584
			mg/pers./d:	0.1029024	0.06783264
kg bw:	70		mg/kg bw/d:	0.00147003	0.00096904
syst. AOEL:	0.009		**% of AOEL:**	**16.3337143**	**10.7670857**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Particle filtering half mask (m/l)	ST 110	0.08	**Im**
Half mask with comb. filter (m/l)	ST 210	0.02	
Particle filtering half mask (appl.)	ST 120	0.08	**Ia**
Half mask with comb. filter (appl.)	ST 220	0.02	
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**
Half mask (appl.)	ST 120 / 220	0.8	**Da/c**
Broad-brimmed headgear (appl.: high crops)	**SS 420**	**0.5**	
Hood and visor (appl.: high crops)	SS 520	0.05	
Protective garment + sturdy footwear (appl.)	**SS 220**	**0.05**	**Da/b**

B.6.14.1.2.4 Manual upward spraying in pome fruits, stone fruits and citrus without and with PPE (gloves during mixing/loading)

= HIGH CROP HAND HELD (HCHH) = **(at BBA)**

Treated area per day	**A =**	***0.45***	**ha/d**	**(1 ha/d)**
Use rate	**R =**	***0.144***	**kg a.i./ha**	

Mixing/loading of the product [mg/person per kg a.i.]				Appl. of the spray [mg/pers. per kg a.i.]	
	liquid	solid: WP	solid: WG	I*a = 0,3	D*a/c = 4,8
I*m	0.05	0.8	0.02	D*a/h = 10,6	D*a/b = 25
D*m/h	205	50	21		

Estimated inhalation exposure:

Im = I*m x R x A	***0.05***	0.144	0.45	0.00324	mg/pers. x d
Ia = I*a x R x A	0.3	0.144	0.45	0.01944	mg/pers. x d
			I, in total =	**0.02268**	**mg/pers. x d**

Estimated dermal exposure:

Dm/h = D*m/h x R x A	***205***	0.144	0.45	13.284	mg/pers. x d
Da/h = D*a/h x R x A	10.6	0.144	0.45	0.68688	mg/pers. x d
Da/c = D*a/c x R x A	4.8	0.144	0.45	0.31104	mg/pers. x d
Da/b = D*a/b x R x A	25	0.144	0.45	1.62	mg/pers. x d
			D, in total =	**15.90192**	**mg/pers. x d**

Estimated inh. exp.		**PPE**	**factor**		
Im =	0.00324	-	1		0.00324 mg/pers. x d
Ia =	0.01944	-	1		0.01944 mg/pers. x d
					0.02268 mg/pers. x d

Estimated derm. exp.

Dm/h =	13.284	SS 110	0.01		0.13284 mg/pers. x d
Da/h =	0.68688		1		0.68688 mg/pers. x d
Da/c =	0.31104		1		0.31104 mg/pers. x d
Da/b =	1.62		1		1.62 mg/pers. x d
					2.75076 mg/pers. x d

		Estimated exposure		**Systemic exposure**	
	abs. rate	**without PPE**	**with PPE**	**without PPE**	**with PPE**
Inhalation: m/l	100%	0.00324	0.00324	0.00324	0.00324
Inhalation: appl.	100%	0.01944	0.01944	0.01944	0.01944
Dermal: m/l	0.4%	13.284	0.13284	0.053136	0.00053136
Dermal: appl.	3%	2.61792	2.61792	0.0785376	0.0785376
			mg/pers./d:	0.1543536	0.10174896
kg bw:	70		mg/kg bw/d:	0.00220505	0.00145356
syst. AOEL:	0.009		**% of AOEL:**	**24.5005714**	**16.1506286**

Possible PPE: specific instructions	Abbr.	Red.-factor	to lower:
Protective gloves (m/l)	**SS 110**	**0.01**	**Dm/h**
Protective gloves (appl.)	**SS 120**	**0.01**	**Da/h**

B.6.14.1.3 RISK ASSESSMENT FOR OPERATORS

Risk assessment was performed using the 75th percentile from the UK-model (UK-75th) and the geometric mean from the German model (DE-GM).

Table 6.14.1.3-1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE **
Mechanical upward spraying on grapes						
UK- 75th	Respiratory	0.03	0.03	0.54	5	5
	Dermal	0.41	0.35	0.54	75	66
	Total	**0.44**	**0.38**	**0.54**	**81**	**71**
DE- GM	Respiratory	0.01	0.01	0.63	2	2
	Dermal	0.27	0.27	0.63	43	42
	Total	**0.29**	**0.28**	**0.63**	**45**	**44**
Mechanical upward spraying on pome fruits and stone fruits						
UK- 75th	Respiratory	0.03	0.03	0.54	5	5
	Dermal	0.44	0.36	0.54	81	66
	Total	**0.46**	**0.39**	**0.54**	**86**	**72**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	0.41	0.40	0.63	65	63
	Total	**0.43**	**0.42**	**0.63**	**68**	**67**
Mechanical upward spraying on citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	3	3
	Dermal	0.26	0.18	0.54	48	34
	Total	**0.28**	**0.20**	**0.54**	**51**	**37**
DE- GM	Respiratory	0.02	0.02	0.63	3	3
	Dermal	0.41	0.40	0.63	65	63
	Total	**0.43**	**0.42**	**0.63**	**68**	**67**
Manual upward spraying on grapes						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2
	Dermal	0.55	0.32	0.54	102	59
	Total	**0.56**	**0.33**	**0.54**	**105**	**61**

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE **
DE- GM	Respiratory	0.02	0.02	0.63	2	2
	Dermal	0.09	0.05	0.63	14	8
	Total	**0.10**	**0.07**	**0.63**	**16**	**11**
Manual upward spraying on pome fruits and stone fruits						
UK- 75th	Respiratory	0.01	0.01	0.54	2	2
	Dermal	0.55	0.32	0.54	102	59
	Total	**0.56**	**0.33**	**0.54**	**105**	**61**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	0.13	0.08	0.63	21	13
	Total	**0.15**	**0.10**	**0.63**	**25**	**16**
Manual upward spraying on citrus						
UK- 75th	Respiratory	0.01	0.01	0.54	1	1
	Dermal	0.41	0.17	0.54	75	32
	Total	**0.41**	**0.18**	**0.54**	**76**	**33**
DE- GM	Respiratory	0.02	0.02	0.63	4	4
	Dermal	0.13	0.08	0.63	21	13
	Total	**0.15**	**0.10**	**0.63**	**25**	**16**

* The AOEL systemic is 0.54 mg/day, using a body weight of 60 kg for the UK-model, and 0.63 mg/day, using a body weight of 70 kg for the German model.

** PPE: only gloves during mixing and loading

B.6.14.2 Bystander exposure (IIIA 7.2.2)

Input data

Application rate : 0.096 – 0.144 kg a.s./ha

Spray volume : 1000 – 3000 L/ha

Dermal exposure : 5% of the application rate (kg/ha) on an body surface of 2 m^2 and dermal absorption of 3%

Inhalation exposure : 0.06 mL spraying liquid per m^3, a duration of 1 hour, a ventilation rate of 1.25 m^3/hour, and inhalation absorption of 100%.

B.6.14.2.1 EXPOSURE ESTIMATES WITH EUROPOEM II

The values represent the 90 th percentile exposure values for bystanders.

B.6.14.2.1.1 UPWARD SPRAYING ON GRAPES

BYSTANDER EXPOSURE **EUROPOEM II MODEL**

form	Envidor SC 240		**Outdoor application**	
as	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.096	kg a.s. / ha	summary of intended uses
SV	Spray volume	1000	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.06	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0072**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.05		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**0.96**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	3	%	
	AOEL	0.63	mg a.s./ day	based on 70 kg bw

	Without PPE	
Internal exposure	[mg a.s./ day]	
Inhalation	0.0072	IE(int) = IE x (IA/100)
Dermal	0.029	DE(int) = DE x (DA/100)
Total	**0.036**	**sum**
% AOEL		

Inhalation	1.1	%AOEL = 100 x IE(int) / AOEL
Dermal	4.6	%AOEL = 100 x DE(int) / AOEL
Total	**6**	**sum**

B.6.14.2.1.2 UPWARD SPRAYING ON POME FRUITS, STONE FRUITS OR CITRUS

BYSTANDER EXPOSURE		EUROPOEM II MODEL		
form	Envidor SC 240	**Outdoor application**		
as	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
SPRAYING Process outdoor				
AR	Application rate	0.144	kg a.s. / ha	summary of intended uses
SV	Spray volume	1500	L / ha	summary of intended uses
Inhalation Exposure				without PPE
	Default value			
SE	Surrogate Exposure Value	0.06	mL / m3	downwards: 0.03; upwards: 0.06 (EUROPOEM II)
T	Time of exposure	1	h	most probable estimation
RR	Respiratory rate	1.25	m3 / h	default
Inhalation Exposure		**0.0072**	mg a.s. / day	IE = (ARx1000/SV)xSExTxRR
Dermal Exposure				
	Default value			
SE	Surrogate Exposure Value	0.05		downwards: 0.005; upwards with leaves: 0.05; upward without leaves: 0.15 (EUROPOEM II)
SA	Surface area bystander	2	m2	EUROPOEM II
Dermal Exposure		**1.44**	mg a.s./ day	DE = SE xSA X (AR x 100)
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	3	%	
	AOEL	0.63	mg a.s./ day	based on 70 kg bw

	Without PPE	
Internal exposure	[mg a.s./ day]	
Inhalation	0.0072	IE(int) = IE x (IA/100)
Dermal	0.043	DE(int) = DE x (DA/100)
Total	**0.050**	**sum**
% AOEL		
Inhalation	1.1	%AOEL = 100 x IE(int) / AOEL
Dermal	6.9	%AOEL = 100 x DE(int) / AOEL
Total	**8**	**sum**

B.6.14.2.2 RISK ASSESSMENT FOR BYSTANDERS

TABLE 6.14.2.2-1 BYSTANDER INTERNAL EXPOSURE AND RISK ASSESSMENT

Route	Estimated internal exposure (mg a.s./day)	AOEL systemic * (mg a.s./day)	%AOEL
Exposure during upward spraying on grapes			
Respiratory	0.0072	0.63	1
Dermal	0.029	0.63	5
Total	**0.036**	**0.63**	**6**
Exposure during upward spraying on pome fruits, stone fruits or citrus			
Respiratory	0.0072	0.63	1
Dermal	0.043	0.63	7
Total	**0.050**	**0.63**	**8**

* Assuming a body weight of 70 kg

B.6.14.3 Worker exposure (IIIA 7.2.3)

Re-entry activities in grapes

Input data

Application rate : 0.096 kg a.s./ha

Dermal exposure (transfer coefficient) : 0.45 m^2/hour

Duration of activities : 6 hours

Re-entry activities in pome fruits, stone fruits or citrus

Input data

Application rate : 0.144 kg a.s./ha

Dermal exposure (transfer coefficient) : 0.45 m^2/hour

Duration of activities : 6 hours

B.6.14.3.1 Exposure estimates with EUROPOEM II

B.6.14.3.1.1 Re-entry activities in grapes, outdoors, based on EUROPOEM II

WORKER EXPOSURE — **EUROPOEM II MODEL**

form: Envidor SC 240
a.s.: spirodiclofen

Re-entry exposure, DFR model

Parameter		Value	Unit	References, comments
Re-entry activities in the field				
AR	Application rate	0.096	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**7.776**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	3	%	
	PPE-factor dermal	0.1		reduction factor
	AOEL	0.63	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	0.233	0.023	DE(int) = DE x (DA/100)
Total	**0.233**	**0.023**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	37	4	%AOEL = 100 x DE(int) / AOEL
Total	**37**	**4**	**sum**

B.6.14.3.1.2 Re-entry activities in pome fruits, stone fruits or citrus, outdoors, based on EUROPOEM II

WORKER EXPOSURE			EUROPOEM II MODEL	
form	Envidor SC 240		**Re-entry exposure, DFR model**	
a.s.	spirodiclofen			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in the field				
AR	Application rate	0.144	kg a.s./ha	summary of intended uses
Worker				
Duration				
T		6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
	no model available	-		
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.45	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**11.664**	mg a.s./ day	DE = DFR x AR x TC x T
Internal exposure				
DA	Dermal Absorption	3	%	
	PPE-factor dermal	0.1		reduction factor
	AOEL	0.63	mg a.s./ day	based on 70 kg bw

	Without PPE	**With PPE**	
Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
Inhalation	-	-	no model available
Dermal	0.350	0.035	DE(int) = DE x (DA/100)
Total	**0.350**	**0.035**	**sum**
% AOEL			
Inhalation	-	-	no model available
Dermal	56	6	%AOEL = 100 x DE(int) / AOEL
Total	**56**	**6**	**sum**

B.6.14.3.2 Risk assessment for workers

Table 6.14.3.2-1 Worker internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE **		without PPE	with PPE **
Re-entry exposure after spraying on grapes						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.233	0.023	0.63	37	4
	Total	**0.233**	**0.023**	**0.63**	**37**	**4**
Re-entry exposure after spraying on pome fruits, stone fruits or citrus						
EURO-POEM II	Respiratory	-	-	-	-	-
	Dermal	0.350	0.035	0.63	56	6
	Total	**0.350**	**0.035**	**0.63**	**56**	**6**

* Assuming a body weight of 70 kg
** **PPE: gloves and coverall; no RPE**
- No model available.

B.6.14.4 Conclusions on risk assessments for operators, bystanders and workers

Safe uses of Envidor SC 240 by operators without PPE were identified for mechanical upward spraying on grapes, pome fruits, stone fruits and citrus using UK-POEM and the German model.

Safe uses of Envidor SC 240 by operators without PPE were identified for manual upward spraying on grapes, pome fruits, stone fruits and citrus using the German model.

Safe uses of Envidor SC 240 by operators without PPE were identified for manual upward spraying on citrus using UK-POEM.

Safe uses of Envidor SC 240 by operators with PPE (protective gloves during mixing/loading) were identified for manual upward spraying on grapes, pome fruits and stone fruits using UK-POEM.

Safe uses for bystanders were identified for mechanical and manual upward spraying on grapes, pome fruits, stone fruits and citrus using the EUROPOEM II 2002 model (90^{th} percentile).

Safe uses for workers without PPE were identified for re-entry activities after application of Envidor SC 240 on grapes, pome fruits, stone fruits and citrus using the EUROPOEM II 2002 model (90^{th} percentile).

European Commission

SPIRODICLOFEN

ADDENDUM

VOLUME 3 (B9)

ANNEX B

Rapporteur Member State: The Netherlands

APRIL 2009

Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

CONTENTS

Evaluation table rev. 2-1 (20.12.2006): Message from EPCO 30 to experts of the sections ecotoxicology and mammalian toxicology: To confirm that new specification from full-scale production is acceptable.

B.9.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

B.9.2.1 Acute toxicity (IIA 8.2)

B.9.2.1.1 Acute toxicity of the new impurities

a) Bruns, E., 2004

A study was performed to assess the acute toxicity of [redacted] to *Danio rerio* under static conditions.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (OJ. No. L383A, 29.12.92) Part C, Method 1 'Acute toxicity for Fish' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 203 'Fish, Acute Toxicity Test'.

Groups of ten fish of the recommended size were exposed to a limit test concentration of nominally 100 mg/L of [redacted] dissolved in water. Auxiliary used to prepare the test medium was an ultra turrax.
Observations were made on the number of dead fish and the incidence of sub-lethal effects after 2, 24, 48, 72 and 96 hours of exposure. The following values were determined:

Time [h]	***LC 0 [mg/L]***
2	≥ 100
24	≥ 100
48	≥ 100
72	≥ 100
96	≥ 100

Highest test concentration resulting in 0% mortality (LC 0 96h): > 100 mg/L

All results are expressed in terms of nominal concentrations. Recovery rates correspond to 95.3% of nominal values at 0 hours, to 96.9% of nominal values at 24 hours, to 96.2% of nominal values at 48 hours, to 98.7% of nominal values at 72 hours and to 96.6% of nominal values at 96 hours, respectively.

Remarks by RMS

The results are used for risk assessment.

b) Bruns, E., 2001

A study was performed to assess the acute toxicity of [redacted] to *Brachydanio rerio* HAMILTON-BUCHANAN under semi-static conditions.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 1 'Acute toxicity for Fish1 which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 203 'Fish, Acute Toxicity Test1.

Groups of ten fish of the recommended size were exposed to range of concentrations, nominally 0.5, 1 and 2 mg/L of [redacted] dissolved in water. Auxiliaries used to prepare the test media were an ultrasonic bath, a magnetic stirrer and folded filters.
Observations were made on the number of dead fish and the incidence of sub-lethal effects after 2, 24, 48, 72 and 96 hours of exposure. The following values were determined:

Time [h]	**LC 0 [mg/L]**
2	$\geq$ 0.32
24	$\geq$ 0.26
48	$\geq$ 0.34
72	$\geq$ 0.39
96	$\geq$ 0.4

Highest test concentration resulting in 0% mortality (LC 0 96h): 0.4 mg/L

All results are expressed in terms of mean measured concentrations. Measured concentrations ranged from 16 - 62% of nominal values in the freshly prepared medium and from 4.5 - 22% of nominal values after 24 hours of exposure.

Remarks by RMS
The results are used for risk assessment.

c) Bruns E., 2004

A study was performed to assess the acute toxicity of [redacted] to *Daphnia magna* STRAUS under static conditions.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 'Acute toxicity for *Daphnia'* which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 202 *'Daphnia sp.,* Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC50 Acute Immobilisation Test'.

The *Daphnia* were exposed to a limit test concentration of nominally 100 mg/L of [redacted] dissolved in water.
Observations were made on the swimming ability and the immobilisation rate, respectively, after 24 and 48 hours of exposure. The following values were determined:

Time [h]	**EC 0 [mg/L]**
24	≥100
48	≥100

Highest test concentration resulting in 0% immobilisation (EC0 48h): >100 mg/L

The results are expressed in terms of nominal concentrations (at 24h and 48h). Recovery rates correspond to 87.4% of nominal values at 0 hours, and to 89.6% of nominal values at 48 hours, respectively.

Remarks by RMS
The results are used for risk assessment.

d) Bruns E., 2004
A study was performed to assess the acute toxicity of [redacted] to *Daphnia magna* STRAUS under static conditions in a closed bottle system.

The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 'Acute toxicity for *Daphnia*' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 202 *'Daphnia sp.*, Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC50 Acute Immobilisation Test'.

The *Daphnia* were exposed to a limit test concentration of nominally 1.9 mg/L of [redacted] dissolved in water. Auxiliaries used to prepare the test media were an ultrasonic bath, a magnetic stirrer and a folded filter.
Observations were made on the swimming ability and the immobilisation rate, respectively, after 24 and 48 hours of exposure. The following values were determined:

Time [h]	**EC 0 [mg/L]**
24	≥ 1.9
48	≥ 0.77

The results are expressed in terms of nominal concentrations (at 24h), and in terms of mean measured concentrations (at 48h). Measured concentrations correspond to 46.8% of nominal values at 0 hours, and to 34.2% of nominal values at 48 hours, respectively.

Remarks by RMS

The results are used for risk assessment.

B.9.2.3 Risk assessment aquatic organisms regarding the new impurities

Summary of the data

Organism Compound	Fish	Daphnia	Algae
Old Material Spirodiclofen	LC_{50} > 58.3 mg/L	EC_{50}> 100 mg/L	EC_{50}> 4.62 mg/L
[redacted]	$LC_0 \geq$ 100 mg/L	$EC_0 \geq$ 100 mg/L	10 > EC_{50}>100 mg/L
N,N-Dimethyl-acetamide	LC_{50} > 500 mg/L	EC_{50} > 500 mg/L	EC_{50} > 500 mg/L
[redacted]	$LC_0 \geq$ 0.4 mg/L	$LC_0 \geq$ 0.77 mg/L	n.a.

n.a., not available,

The data for N,N-Dimethyl-acetamide are originating from material safety data sheets.

The notifier submitted the following statement regarding the risk of the new impurities:

From the provided individual data on the toxicity of the three impurities to aquatic organisms a significant change in the hazard potential of the new material is not evidenced. Data as summarized on material safety data sheets indicate low toxicity for [redacted] *and N,N Dimethylacetamide. EC5o algae of 10-100 mg/L, and E(L)C50 daphnia/fish >100 mg/L were reported for* [redacted]*. N,N Dimethylacetamide's toxicity is published with E(L)C50-values greater than 500 mg/L, hence a contribution to the toxicity of the active pure chemical spirodiclofen by any of these impurities is not anticipated, considering the specified limit of* [redacted] *g/kg.*

The potential contribution of [redacted] *to the overall toxicity of the new material was assessed by taking into consideration toxicity data on fish and daphnia from a related compound,* [redacted]*. It has the identical structure as* [redacted] *but the carboxylic acid is* [redacted]*. As such, this compound is more lipophilic and thus more likely to be taken up into aquatic organisms but, its water solubility is limited (1.9 mg/L, see safety data sheet). Testing up to high doses is therefore hampered by the lack to achieve sufficient test concentrations in water. Up to the maximum tested concentration of ca. 2 mg/L no indication of toxicity to fish and daphnia was found (EC0 daphnia, > 0.4 mg/L, LC0 fish, > 0.77 mg/L). From these aquatic toxicity data a significant change of the hazard potential of the new material to aquatic organisms is not evidenced. However, for* [redacted] *a definite aquatic endpoint is not available and as such, some uncertainty remains. Nevertheless, due to the specified limit of* [redacted] *g/kg of this impurity BCS considers this uncertainty justifiable taking into consideration an approach that is recommended in the Guidance Documents for Aquatic and Terrestrial Ecotoxicology. Being somehow structurally related to spirodiclofen itself* [redacted] *is assumed to be 10 times more toxic than the active compound as suggested for assessing metabolites with unknown toxicity in these guidance documents. As such, the toxicity of the old material, namely L(E)C50 values of > 4.62 mg/L, > 100 mg/L and > 58.3 mg/L for algae, daphnia and fish, respectively, would be "mixed" by a compound being 10 time more toxic but present in a concentration of only* [redacted] *g/kg. The change in the endpoint by such a toxic impurity (e.g. 0.46 mg/L, 10 mg/L, 5.8 mg/L as effects endpoints) can be assessed as minor considering mixed toxicity calculations. It is doubtful that this minor change would be detectable in repeated tests of the new material with aquatic organisms due to the technical variation expected for a lipophilic chemical as spirodiclofen. Under the view that spirodiclofen is of little water solubility and as such, the acute toxicity of the old material needed to be determined by using the formulated product, it is unlikely that such a minor change would be measurable within the frame of the precision of the test system.*

Reaction RMS

The RMS can agree with the statement of the notifier with respect to the risk of the new impurities to aquatic organisms.

B.9.6 Effects on earthworms (IIA 8.4, IIIA 10.6.1)

B.9.6.1 Toxicity

B.9.6.1.1 Toxicity of the active substance with the new impurities

Lührs, U., 2007

In an acute toxicity limit study, earthworms (*Eisenia foetida*) were exposed to Spirodiclofen technical (purity 97.8%) for 14 days in artificial soil.
Spirodiclofen was mixed into the soil at 100, 178, 316, 562 and 1000 mg test item/kg artificial soil (dry weight) to which earthworms *Eisenia fetida* (40 worms per test item group) were exposed for 14 days at 19 - 21°C, light 430 - 780 lux, continuous illumination, initial soil water content 56.3% to 58.1% of the max. water holding capacity, water content at experimental termination 52.7% to 55.0% of the max. water holding capacity; initial pH 6.4, pH 5.9 to 6.2 at experimental termination; Endpoints were mortality and body weight change.

The LC50 was determined to be greater than 1000 mg test item/kg soil. The No Observed Effect Concentration (NOEC) related to mortality and biomass was determined to be 1000 mg Spirodiclofen/kg soil dry weight, *i.e.* the highest tested concentration.

Remarks by RMS
The results are used for risk assessment.

B.9.8 Effects on soil non-target micro-organisms (IIA 8.5, IIIA 10.7)

B.9.8.1 Toxicity

B.9.8.1.1 Toxicity of the active substance (IIA 8.5)

a) Schulz, L., 2007

Data were submitted from a laboratory study of the effect of technical Spirodiclofen (97.6% pure) on respiration in a loamy sand soil (pH 7.0; 1.96% Corg; WHC: 39.17 g/100 g dry soil).

The test was performed in accordance to the OECD guideline 216 (2000).
The nitrogen transformation (NO3-nitrogen production) was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). A comparison of the test item treated soil with a non-treated soil was made; three replicates per treatment and concentration. NH4-nitrogen, N03- and NO2-nitrogen were determined by using the Autoanalyzer II (BRAN+LUEBBE).
Sampling scheme: 0, 7, 14 and 28 days after treatment.

The test concentrations were: control, 0.20 mg test item/kg dry soil (corresponding to an application rate of 148 g test item/ha) and 0.98 mg test item/kg dry soil (corresponding to an application rate of 738 g test item/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm3.

No adverse effects of BAJ 2740 tech. on nitrogen transformation in soil were observed in both test concentrations (0.20 mg/kg dry soil and 0.98 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +1.6 % (test concentration 0.20 mg/kg dry soil) and +1.6 % (test concentration 0.98 mg/kg dry soil) were measured at the end of the 28-day incubation period (see table below).

Table 1: Effects on nitrogen transformation in soil after treatment with BAJ 2740 tech.

Days after application	Control	0.20 mg test item/kg soil dry weight equivalent to 148 g test item/ha		0.98 mg test item/kg soil dry weight equivalent to 738 g test item/ha	
	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	Deviation from control [%][1)]
0	12.0	12.2	+1.9	12.0	+0.3
7	36.4	35.3	-2.9	35.5	-2.4
14	40.7	40.7	-0.2	40.6	-0.4
28	48.4	49.1	+1.6	49.1	+1.6

The calculations were performed with unrounded values
1) based on NO_3-nitrogen production; - = % inhibition; + = % stimulation
* statistically significantly different to control (Student-t-test, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +27.2 %, +43.1 % and +51.2 % at 6.80, 16.00 and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Conclusion

BAJ 2740 tech. caused no adverse effects (deviation from control <25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations equivalent up to a field application rate of 738 g test item/ha (equivalent to 720 g a.i./ha).

Remarks by RMS

The results are used for risk assessment.

b) Schulz, L., 2007

Data were submitted from a laboratory study of the effect of technical Spirodiclofen (97.6% pure) on respiration in a loamy sand soil (pH 7.0; 1.96% Corg; WHC: 39.17 g/100 g dry soil).

The test was performed in accordance to the OECD guideline 217 (2000).
The carbon transformation was determined in soil after addition of glucose. A comparison of the test item treated soil with a non-treated soil was made; three replicates per treatment and concentration. A respirometer system was used to determine the O2-consumption over a period of maximum 24 hours at different sampling intervals.
Sampling scheme: 0, 7, 14 and 28 days after treatment.

The test concentrations were: control, 0.20 mg test item/kg dry soil (corresponding to an application rate of 148 g test item/ha) and 0.98 mg test item/kg dry soil (corresponding to an application rate of 738 g test item/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm3.

No adverse effects of BAJ 2740 tech. on carbon transformation in soil were observed in both test concentrations (0.20 mg/kg dry soil and 0.98 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +1.3 % (test concentration 0.20 mg/kg dry soil) and -1.6 % (test concentration 0.98 mg/kg dry soil) were measured at the end of the 28-day incubation period.
Statistical analysis (Student-t-test, 2-sided, p <0.05) revealed no significant differences for both tested concentrations compared to control 28 days after treatment. See table below.

Table 1: Effects on carbon transformation in soil after treatment with BAJ 2740 tech.

Days after application	Control	0.20 mg test item/kg soil dry weight equivalent to 148 g test item/ha		0.98 mg test item/kg soil dry weight equivalent to 738 g test item/ha	
	O_2 consumption [mg/kg soil d.w./h]	O_2 consumption [mg/kg soil d.w./h]	Deviation from control [%][1]	O_2 consumption [mg/kg soil d.w./h]	Deviation from control [%][1]
0	10.47	10.82	+3.3*	10.54	+0.7
7	9.69	9.56	-1.4	9.81	+1.2
14	8.99	8.94	-0.6	8.93	-0.7
28	8.54	8.64	+1.3	8.40	-1.6

The calculations were performed with unrounded values.
1) based on O_2 consumption; - = % inhibition; + = % stimulation
* statistically significantly different to control (Student-t-test, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused an inhibition of carbon transformation of -38.8 % and -38.1 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application.

Conclusion

BAJ 2740 tech. caused no adverse effects (deviation from control <25 %, OECD 217) on the soil carbon
transformation (measured as oxygen consumption) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations equivalent up to a field application rate of 738 g test item/ha (equivalent to 720 g a.i./ha).

Remarks by RMS

The results are used for risk assessment.

B.9.8.2 Risk assessment of the active substance with the new impurities on soil organisms

The notifier submitted the following statement with respect to the risk of the new impurities:

Old material

Spirodiclofen as manufactured according to the old specification is not toxic to earthworm. The old material has been tested with > 1000 mg/kg soil dryweight as the acute LCso value (Table II). Effects on soil micro-organisms were equally without concern, there was no adverse effect up to a rate of 0.98 kg a.s./ha on both carbon mineralization and nitrogen turnover. Within the nitrogen mineralization test a transient increase in nitrate formation rate in comparison to the untreated control was observed at

second sampling. This increase is not considered as an adverse effect to soil function and had disappeared 42 days past application.
Overall, spirodiclofen is of low hazard to soil vitality on both species and functional level. Toxicity-exposure calculations based upon predicted environmental concentrations in soil from uses in top fruits as notified within the Annex I listing process are far above the trigger values laid down in 91/414/EEC. There is little concern about an unacceptable risk to soil organisms due to the low toxicity of spirodiclofen to soil organisms.

Equivalence of the new material

The new material, containing the three impurities [redacted] *N,Ndimethylacetamide and* [redacted] *in the maximum specified limit was tested in an acute test on earthworm following OECD Testing Guideline 207. In order to approve the low toxicity to earthworm as has been found for the old material, a dose-response test with 5 concentrations and untreated control (0-100-178-316-562-1000 mg a.s./kg soil d.w.) was undertaken. No mortality was observed within the 14 days of experimental time in any of the treatment groups. As such, the LC50 is greater than 1000 mg a.s./kg soil dry weight. Equally to the old material, the no observed-effect concentration (NOEC) is 1000 mg a.s./kg soil d.w. and the LOEC is > 1000 mg a.s./kg soil d.w. Therefore, both the old material and the technical spirodiclofen in its new composition are equally untoxic to earthworms.*
Earthworm has been tested as a potentially sensitive species for the change in composition due to the presence of the impurity N,N dimethylacetamide. For this compound screening data are available in the public indicating some intrinsic toxicity to earthworm. As the new material spirodiclofen has been proven to be untoxic to earthworms no concern remains for the presence of this impurity in the new material within the specified limit ([redacted]g/kg). Its concentration in the material is low from which a change in the ecotoxicity had not been anticipated. This has been proven by the test results as outlined above. As such, a change of the toxicity to soil macro-organisms is not evidenced as shown by the absence of toxicity to earthworm.
A toxicity-exposure calculation can be performed as has been done with the old material, to analyse whether the new source could result in a risk for soil macro-organisms. The data are presented in Table III. Based upon the low toxicity the TER is very high, a factor of 500 above the trigger value to be
considered. This indicates that the risk to soil macro-organisms is very low as has been approved also for the old material. Therefore, the ecotoxicological profile of the new material spirodiclofen is considered unchanged.

The effect of the new material on nitrogen and carbon transformation was tested applying a rate corresponding to the single and 10 fold treatment rate in agricultural use (0.2 mg/kg soil dw and 0.98 mg/kg soil dw, [5][6], Table II). As obtained already with the former specified material, there were no adverse effects in both tests at any concentration. The measured deviation in nitrogen and carbon transformation by exposure to the new material was negligible with 1.6 % at day 28. The maximum deviation from the untreated control in carbon mineralization was +3.3% at day 0 at a concentration of 0.2 mg/kg spirodiclofen showing not treatment-related effect (the measured deviation at higher dose level was 0.7%). The maximum deviation in the nitrogen transformation test was -2.9% at the lower dose at day 7 and is equally considered irrelevant. As such, no new quality in the toxicity to soil micro-organisms of the new material has been obtained.

Overall, the data are in agreement with expectations. Even assuming a higher toxicity than the active chemical spirodiclofen itself as is suggested for metabolites according to prevailing Guidance Documents of Ecotoxicology, the low level at which new impurities are specified counteract a significant change in the endpoints. Moreover, for [redacted] *it is assumed that its toxicity had already been covered in the soil studies conducted earlier with the old material because the pure active compound spirodiclofen* [redacted] *Considering the* [redacted] *days, the maximum specified concentration of* [redacted] *would have been* [redacted] *As such, the effects endpoints determined for soil organisms with the old material are highly likely to include already the potential effects of* [redacted]

Conclusion

In summary, the results from the tests on earthworm and soil micro-organisms do not evidence a relevant change that raises a concern. The new source resulted in similar endpoints for earthworm and soil microorganisms as the old technical material. Both technical ingredients as manufactured are non-toxic to earthworm with an LCso value of > 1000 mg a.s./kg soil d.w. TERs calculated based upon the predicted exposure concentrations from uses in top fruits show that there is no concern and even 10 fold higher treatment rates would be within a comfortable margin of safety based uponthe overall low toxicity of both the old and new material.

Also, nitrification and carbon transformation as indicators for a functioning soil microbial population were not negatively influenced by the new material.

Therefore, the new impurities as specified in their limits have not shown any influence on the overall ecotoxicity of spirodiclofen techn. The new studies show, that the ecotoxicological profile of the old material will be reflected in the new spirodiclofen and, both materials can be considered as ecotoxicologically equivalent.

Reaction RMS

The RMS can agree with the statement of the notifier with respect to the risk of the new impurities to soil organisms.

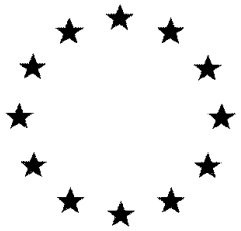
Overall conclusion regarding the new impurities

Based on the submitted studies on the toxicity of the active substance (together with the new impurities) and the new impurities itself for aquatic organisms and soil organisms it can be concluded that the new impurities are not of ecotoxicological significance.

B.9.11 References relied on

Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA, 8.2.1/ 01	Bruns, E.	2004	[redacted], Acute fish toxicity [redacted] Report No.: 1316A/04F/MO-05-000063 GLP, unpublished	Y	BAY
IIA, 8.2.1/ 02	Bruns, E.	2001	[redacted], Acute fish toxicity, [redacted], Report No. 1105 N/01 F/MO-02-001594 GLP, unpublished	Y	BAY
IIA, 8.2.4/ 01	Bruns, E.	2004	[redacted] Acute daphnia toxicity Bayer Industry Services GmbH & Co.OHG, Report No.: 1316N/04D/MO-05-000076 GLP, unpublished	Y	BAY
IIA, 8.2.4/ 02	Bruns, E.	2004	[redacted], Acute daphniatoxicity, Bayer AG, Report No. 1105 N/01 D/MO-02-001593 GLP, unpublished	Y	BAY
IIA, 8.4.1/ 01	Lührs, U.	2007	Spirodiclofen: Acute toxicity (14 days) to the earthworm *Eisenia fetida* in artificial soil BCS-Report-No. M-284516-01-1 GLP, unpublished	Y	BAY
IIA, 8.5.1/ 01	Schulz, L.	2007	Effects of BAJ 2740 tech. on the activity of soil microflora (Nitrogen transformation test) BCS-Report-No. M-284463-01-1 GLP, unpublished	Y	BAY
IIA, 8.5.1/ 02	Schulz, L.	2007	Effects of BAJ 2740 tech. on the activity of soil microflora (Carbon transformation test) BCS-Report-No. M-284457-01-1 GLP, unpublished	Y	BAY

European Commission



SPIRODICLOFEN

ADDENDUM B2 B5

EPCO-MEETING 30

PRAPeR 66

Rapporteur Member State: The Netherlands

June 2005
revised April 2009 (revisions in blue)
revised June 2009(revisions in purple)

Draft Assessment Report and Proposed Decision of The Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC

CONTENTS

Volume 3

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

The notifier submitted studies of the water solubility and partition co-efficient of spirodiclofen at pH 7 to full-fill data requirement 1.3. In order to address open point 1.7 of the Evaluation Table version 2-1 of 20.12.2006, reports with spectral data of the relevant impurities BAJ-2740 enol and N,N-dimethylacetamide were submitted.

B.2.2 Physical, chemical and technical properties of the plant protection products (Annex IIIA 2)

In order to address open point 1.5 of the Evaluation Table version 2-1 of 20.12.2006, a report with a storage stability study of Spirodiclofen 240 SC in HDPE (2 weeks, 54°C) was submitted.

B.2.4 References relied on

References for the active substance

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No., GLP or GEP status,	Data Protection Claimed Y/N	Owner
B.2.1.11 (IIA 2.6)	Bogdoll, B., Strunk, B.	2005	Spirodiclofen, BAAJ 2740 (AE 1344097) – Water solubility at pH 7 (column elution method), Generated by: Bayer AG Report N°: MO-05-007603 Source: Bayer AG Date: 27 April 2005 GLP; Unpublished	Y	Bayer AG
B.2.1.13 (IIA 2.8)	Bogdoll, B., Wiche, A.	2005	Spirodiclofen, BAAJ 2740 (AE 1344097) – Partition coefficient 1-octanol/water at pH 7, Generated by: Bayer AG Report N°: MO-05-007606 Source: Bayer AG Date: 27 April 2005 GLP; Unpublished	Y	Bayer AG
B.2.1.10 (IIA 2.5)	Schmidt M. & Rüngeler W.	2008a	Spectral data set of BAJ 2740-enol reference material, Generated by: Bayer AG Report N°: M-302304-01-1 Source: Bayer AG Date: 06 June 2008 GLP; Unpublished	Y	Bayer AG

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No., GLP or GEP status,	Data Protection Claimed Y/N	Owner
B.2.1.10 (IIA 2.5)	Schmidt M. & Rüngeler W.	2008b	Spectral data set of N,N-dimethylacetamide, Generated by: Bayer AG Report N°: M-302307-01-1 Source: Bayer AG Date: 06 June 2008 GLP; Unpublished	Y	Bayer AG
B.2.2.14 (IIIA 2.7)	Güldner W. & Hoppe M.	2008	Determination of spirodiclofen and quantification of the impurities N,N-dimethylacetamide and BAJ 2740-enol before and after storage at elevated temperature in Spirodiclofen SC 240 (240 g/L), Generated by: Bayer AG Report N°: M-304264-01-1 Source: Bayer AG Date: 15-July 2008 GLP; Unpublished	Y	Bayer AG

Table B.2.1.1 Summary of the physical and chemical properties of the active substance (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise)

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.11 (IIA 2.6)	Solubility in water	99.2 %	Guideline 92/69/EEC, appendix A.6 and OECD-guideline 105, using the column elution method.	At pH 7 and 20 °C: 0.19 mg/L = 190 µg/L		Bogdoll, B.; Strunk, B., 2005
B.2.1.13 (IIA 2.8)	Partition co-efficient	99.2 %	The "flask-shaking" method described in guideline OECD 107 and 92/69/EEC, appendix, part A.8.	Log Pow = 5.1 at 23 °C and pH 7.		Bogdoll, B.; Wiche, A., 2005

Table B.2.1.2 Summary of the physical and chemical properties of impurity BAJ 2740-enol (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.10 (IIA 2.5)	Spectra	99.9 %		IR-,1H-NMR, 13C-NMR, MS- (LC-MS/ESI$^+$) and UV–spectra were submitted. UV spectrum: Solvent: acetonitrile Maximum ε (L/mol/cm) 217 nm 19123 244 nm 11356 Solvent: acetonitrile / pH 2 Maximum ε (L/mol/cm) 202 nm 33419 219 nm 19925 241 nm 12223 Solvent: acetonitrile / pH 10 Maximum ε (L/mol/cm) 252 nm 16263 303 nm 9561	The study was conducted under GLP.	Schmidt M. and Rüngeler W., 2008a

Table B.2.1.3 Summary of the physical and chemical properties of impurity N,N-dimethylacetamide (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise).

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.10 (IIA 2.5)	Spectra	99.9 %		IR-,1H-NMR, 13C-NMR, MS- (LC-MS/ESI$^+$) and UV–spectra were submitted. UV spectrum: Solvent: acetonitrile No maxima detectable Solvent: acetonitrile / pH 2 Maximum ε (L/mol/cm) 202 nm 6943 Solvent: acetonitrile / pH 10 No maxima detectable	The study was conducted under GLP.	Schmidt M. and Rüngeler W., 2008b

Table B.2.2.4 Summary of the physical and chemical properties of the plant protection product

Product name: BAJ 2740 SC240

B.2.2.14 (IIIA 2.7)	Storage stability	CIPAC Method MT 46 and GIFAP monograph 17	Following storage of Spirodiclofen 240 SC for 2 weeks at 54°C in HDPE, the a.s. content (23.1% before and after storage) and the content of the impurity N,N-dimethylacetamide (<0.05% before and after storage) were unchanged. The content of the impurity BAJ 2740-enol was <0.05% before storage and 0.05% after storage. The package was found to be stable during the test.	The study was conducted under GLP. The analytical method was HPLC/UV method AM011108MF1. This method was evaluated in confidential information, revised Addendum to Volume 4, April 2009. Evaluation established that the LOQ for BAJ 2740-enol should be 0.08% instead of the claimed 0.05%. The chromatogram of the stored sample showed no discernible peak for BAJ 27490-enol. The result for this impurity should therefore be stated as follows: The content of the impurity BAJ 2740-enol was <0.08% before and after storage.	Güldner W. and Hoppe M., 2008

B.5 Methods of analysis

B.5.1 Analytical methods for formulation analysis

B.5.1.1 Methods for the determination of pure active substance in the active substance as manufactured and in formulated products

The analytical method AM001404MP1 is used for the determination of the a.s. in the technical material.

B.5.1.2 Description of analytical methods for the determination of impurities

~~Confidential information, see revised Addendum to Volume 4, April 2009~~

The impurities N,N-dimethylacetamide and BAJ 2740-enol are relevant impurities. A HPLC-UV method to analyse these two impurities (and the active substance spirodiclofen) in the plant protection product was submitted (Zitzmann W., 2008; Odendahl A. and Zitzmann W., 2008), to address open point 1.6 of the Evaluation Table version 2-1 of 20.12.2006.

Method AM011108MF1: An amount of sample containing 30-100 mg of spirodiclofen was dissolved in acetonitrile. Spirodiclofen and the byproducts BAJ 2740-enol and N,N-dimethylacetamide were determined by reversed phase HPLC with UV detection at 210 nm. The identity of the peaks was confirmed by DAD spectra. The validation was performed on Spirodiclofen SC 240 and is summarized in Table below. The batch of Spirodiclofen 240 SC used for validation was shown to contain no measurable amounts of the byproducts BAJ 2740-enol and N,N-dimethylacetamide. The repeatability and recovery determinations were therefore performed with Spirodiclofen 240 SC fortified with each of the byproducts.

Validation for the analytical method to determine Spirodiclofen and the byproducts BAJ 2740-enol and N,N-dimethylacetamide

	Analyte	Method	Linearity	Precision – repeatability (RSD)	Accuracy (%)	LOQ	Interference
1	Spirodiclofen	HPLC/UV	50-150% of expected: R = 0.9999	RSD 0.59% (n=6)	Addition at 6 levels (n=1 per level) in range 10-36% w/w: mean recovery 100.43 %	-	No
2	N,N-dimethylacetamide	HPLC/UV	About 0.11-0.40% w/w in product: R = 0.9991	RSD 4.56% (n=6 at measured level of 0.05% w/w in product); acceptable, RSD only slightly exceeds RSD_r(max) (4.21%)	Addition at 3 levels (n=1 per level) in range 0.05-0.22% w/w in product: mean recovery 98.48%	0.05%	No

	Analyte	Method	Linearity	Precision – repeatability (RSD)	Accuracy (%)	LOQ	Interference
3	BAJ 2740-enol	HPLC/ UV	About 0.10-0.48% w/w in product: R = 0.9999	RSD 2.51% (n=6 at measured level of 0.06% w/w in product)	Addition at 3 levels (n=1 per level) in range 0.08-0.20% w/w in product: mean recovery 113.06%	0.08%	No

The submitted validation was acceptable for spirodiclofen. It also supported the proposed LOQ of 0.05% w/w for N,N-dimethylacetamide. The author of the report proposed that the LOQ for BAJ 2740-enol be 0.05% based on signal/noise ratio. It should be noted that the fortification level for the precision-repeatability measurement (RSD 2.51% at measured level of about 0.06% w/w) was not reported and hence this measurement was suitable for determination of repeatability only, not recovery. As a consequence, the lowest level with full validation for BA2740-enol was 0.08% w/w. In the chromatogram of the signal/noise ratio at the proposed LOD (0.03%) for BA2740-enol an overlapping peak eluted just after BAJ 2740-enol, and this peak was probably responsible for the asymmetry of the BAJ 2740-enol peak observed in the chromatogram of the signal/noise ratio at the proposed LOQ (0.05%). The inclusion of this peak in the peak area of the BAJ 2740-enol peak during the recovery determinations was possibly responsible for the fact that the recovery at 0.08-0.20% w/w (110-117%, mean 113.06%) exceeded 100%. At fortification levels below 0.08% recovery might no longer be acceptable since the relative contribution of the interfering peak will be higher. Therefore the LOQ for BAJ 2740-enol was set at 0.08% by the RMS.

Assuming that the density of the plant protection product is 1.085 g/mL (volume 3, B.2.2.13), the spirodiclofen content in the plant protection product (240 g a.s./L) is equivalent to 22.1% w/w. The LOQ of 0.05% w/w and 0.08% w/w, respectively, for N,N-dimethylacetamide and BAJ 2740-enol, would correspond with levels of 0.23% w/w and 0.36% w/w in spirodiclofen technical. On the basis of the results of the analysis of the five full scale production batches, maximum limits of [redacted]% and [redacted]% w/w for N,N-dimethylacetamide and BAJ 2740-enol, respectively, have been proposed. The methods of analysis for the byproducts in the plant protection products should be capable to determine each byproduct at the maximum level. Whether this is the case cannot be established at present, since no maximum levels for both byproducts have been agreed yet (List of Endpoints of EFSA Scientific Report (2007) 104).

B.5.2 Analytical methods for plants, plant products, foodstuffs and feedingstuffs

It has been noted in section 1 of the evaluation table that depending on the outcome of the residue expert meeting (with reference to open point 3.3 of the evaluation table), it could be necessary to require an enforcement method for food of animal origin, see also reporting table 1 (17)

For animal products spirodiclofen-enol is the residue of concern. An enforcement method to determine

spirodiclofen-enol (M01) in cattle products (meat, milk, kidney, liver, fat) should be provided, since deuterated internal standards are not commonly available (Method 109720, see B.5.2.4 in the monograph) and implementation in an multiresidue method was not tested (Enforcement method 00086/M030 [extended revision of DFG Method S19], see B.5.2.1 in the monograph).

A new method (Zimmer (2005) MO-05-005229) for enforcement of animal matrices (muscle, milk, liver, fat) for parent and BAJ 2740-enol and an ILV (Bacher (2005), MO-05-005724) are submitted.

STUDY 1

reference	:	Zimmer, D.; Kuppels, U; Philipowski, C.; 2005 (MO-05-005229)	GLP statement	:	yes
type of study	:	Residue analytical method 00919 for the determination of residues of BAJ 2740 (AE 1344097) and its metabolite BAJ 2510 (AE1344098) in/on animal tissues and milk by HPLC-MS/MS	guideline	:	EU guidelines SANCO/825/00 and SANCO/3029/99
year of execution	:	2005	acceptability	:	yes
test substance	:	Spirodiclofen (BAJ 2740), purity 98.2 % (m/m) BAJ 2510, purity 99.9 % (m/m)			

Method summary

The analytical method presented was developed and validated to determine the active ingredient (a.i.) BAJ 2740 and its major metabolite (BAJ 2510) in milk and animal tissues obtained from bovine: fat, muscle, kidney and liver. BAJ 2740 and the enol BAJ 2510 are extracted by maceration in acetonitrile/water (4/1, v/v) containing 0.1 % formic acid.

An aliquot of the analytical solution is injected into a high-performance liquid chromatograph, chromatographed by gradient elution under reversed phase conditions with a methanol/aqueous ammonium formiate/formic acid eluent on a silica based Cis column and finally detected by tandem mass spectrometry (MS/MS) with electrospray ionisation (LC-ESI-MS/MS). BAJ 2740 and the metabolite BAJ 2510 are quantified against external matrixmatched standards. For tandem mass spectrometric quantitation the ion transitions (MRM) from [M+H]+ at m/z 411 to the quantifier fragment ion m/z 313 for BAJ 2740 and from [M+H]+ at m/z 313 to the quantifier fragment ion m/z 213 for the Enol BAJ 2510 are used.

For BAJ 2740 the ion chromatograms of the MRM-transitions from [M+H]+ at m/z 411 to the 3 fragment ions m/z 295, 213 and 157 are summarized, since the intensity of only one of these transitions was not sufficient to confirm the analyte at the LOQ. The single confirmatory fragment ion for BAJ 2510 is m/z 231, which gives a sufficiently high intensity even at the LOQ.

Validation data for the method for the determination of spirodiclofen and its metabolite BAJ 2510 in products of animal origin

Recovery

The results of the recovery experiments are given in tables B.5.2a for spirodiclofen and in table B.5.2c for BAJ 2510.

Similar recovery data were calculated with the confirmatory method, the details are shown in tables B.5.2b for spirodiclofen and in table B.5.2d for BAJ 2510.

Linearity

The linearity of the LC/MS/MS-method was tested by triplicate analyses of both, matrix-matched standards prepared of bovine (fat, kidney, liver, muscle and milk) and standards in solvent.

The correlation coefficients of the 1/x weighted linear regression were in all cases > 0.999 and the y-axis intercepts were well below 10% of the LOQ.

Specificity

The high selectivity (specificity) of the method resulted from the LC separation in combination with the very selective tandem mass spectrometric (MS/MS) detection. For confirmation of the detection of the analytes additional fragment ions are monitored.

From each sample material, up to two untreated control samples were examined. All determined residues of BAJ 2740 and BAJ 2510 were well below 30% of the LOQ level.

Limit of Quantitation

The limit of quantitation (LOQ) was defined as the lowest validated fortification level where a mean recovery within the range of 70 to 110% and an RSD of < 20 % could be obtained, provided that blank values are below 30% of this level. The LOQ was established at 0.005 mg/kg for milk, 0.01 mg/kg for fat and muscle, and 0.05 mg/kg for kidney and liver, respectively.

Table B.5.2a Recoveries and Repeatability for BAJ 2740 (Quantitation Method)

Sample Material		Fortification Level (mg/kg)	Recovery Rates (%) BAJ 2740 m/z 313 Quantifier Ion Single Values					Mean	RSD (%)
Bovine	Milk	0.005	108	108	106	96	116	107	6.7
		0.005*	100	91	121	88	115	103	14.1
		0.05	106	111	122	92	90	104	12.8
						overall mean		**105**	**10.8**
Bovine	Fat	0.01	94	100	101	96	105	99	**4.4**
		0.10	89	91	95	100	100	95	5.3
						overall mean		**97**	**5.1**
Bovine	Muscle	0.01	87	87	76	87	94	86	7.5
		0.10	87	84	80	86	80	83	3.9
						overall mean		**85**	**6.0**
Bovine	Kidney	0.05	96	97	99	99	96	97	1.6
		0.50	103	101	102	109	106	104	3.1
						overall mean		**101**	**4.3**
Bovine	Liver	0.05	94	98	95	97	94	96	1.9
		0.50	103	104	100	100	101	102	1.8
						overall mean		**99**	**3.6**
Total Number of Recoveries:						**55**			
Overall Mean:								**98**	
Overall RSD:									**9.9**

Table B.5.2b Recoveries and Repeatability for BAJ 2740 (Confirmation Method)

Sample Material		Fortification Level (mg/kg)	Recovery Rates (%) BAJ 2740 Confirmatory Ions Sum of m/z 295.1&213.0&157.1 Single Values					Mean	RSD (%)
Bovine	Milk	0.005	93	114	83	86	126*	100	18.7
		0.05	115	126*	117	110	92	112	11.2
						overall mean		**106**	**15.3**
Bovine	Fat	0.01	83	84	92	97	90	89	6.5
		0.10	85	92	96	97	99	94	5.9
						overall mean		**92**	**6.4**
Bovine	Muscle	0.01	84	82	93	95	99	91	8.1
		0.10	88	84	85	84	74	83	6.4
						overall mean		**87**	**8.3**
Bovine	Kidney	0.05	95	100	101	101	110	101	5.3
		0.50	103	101	103	103	105	103	1.4
						overall mean		**102**	**3.7**
Bovine	Liver	0.05	89	84	100	112	80	93	14.0
		0.50	98	104	101	99	95	99	3.4
						overall mean		**96**	**9.9**
Total Number of Recoveries:						**50**			
Overall Mean:								**97**	
Overall RSD:									**12.0**

Table B.5.2c Recoveries and Repeatability for BAJ 2510 (Quantitation Method)

Sample Material		Fortification Level (mg/kg)	Recovery Rates (%) BAJ 2510 Quantifier Ion m/z 213 Single Values					Mean	RSD (%)
Bovine	Milk	0.005	98	100	98	94	102	98	3.0
		0.005*	111	90	86	90	107	97	11.7
		0.05	103	102	103	99	99	101	2.0
						overall mean		**99**	**6.7**
Bovine	Fat	0.01	96	96	96	105	94	97	4.5
		0.10	99	98	99	102	103	100	2.2
						overall mean		**99**	**3.6**
Bovine	Muscle	0.01	96	89	88	86	87	89	4.4
		0.10	95	92	94	91	96	94	2.2
						overall mean		**91**	**4.1**
Bovine	Kidney	0.05	87	85	87	93	97	90	5.6
		0.50	94	96	95	98	98	96	1.9
						overall mean		**93**	**5.3**
Bovine	Liver	0.05	91	106	104	95	103	100	6.5
		0.50	101	100	101	99	100	100	0.8
						overall mean		**100**	**4.3**
Total Number of Recoveries:						**55**			
Overall Mean:								**97**	
Overall RSD:									**6.1**

Table B.5.2d Recoveries and Repeatability for BAJ 2510 (Confirmation Method)

Sample Material		Fortification Level (mg/kg)	Recovery Rates (%) BAJ 2510 Confirmatory Ion m/z 231 Single Values					Mean	RSD (%)
Bovine	Milk	0.005	94	125	97	77	104	99	17.5
Sum	m/z 231.0&185.0&156.9	0.05	101	103	105	97	99	101	3.1
						overall mean		**100**	**11.8**
Bovine	Fat	0.01	93	88	91	97	97	93	4.2
	m/z 231 only	0.10	98	99	98	101	101	99	1.5
						overall mean		**96**	**4.5**
Bovine	Fat 2.	0.01	101	100	104	99	105	102	2.5
Sum	m/z 231.0&185.0&156.9	0.10	98	100	103	104	104	102	2.6
						overall mean		**102**	**2.4**
Bovine	Muscle	0.01	88	96	100	89	88	92	6.0
		0.10	92	93	95	97	100	95	3.4
						overall mean		**94**	**4.9**
Bovine	Kidney	0.05	87	94	92	97	82	90	6.6
		0.50	94	93	97	98	99	96	2.7
						overall mean		**93**	**5.7**
Bovine	Liver	0.05	100	67	99	74	100	88	18.4
	m/z 231 only	0.50	96	98	99	99	101	99	1.8
						overall mean		**93**	**13.1**
Bovine	Liver	0.05	95	107	95	84	101	96	8.9
Sum	m/z 231.0&185.0&156.9	0.50	98	98	100	97	100	99	1.4
						overall mean		**98**	**6.0**
Total Number of Recoveries:						**70**			
Overall Mean:								**97**	
Overall RSD:									**8.2**

Accuracy

The overall accuracy of the quantitation method was 98 % for spirodiclofen.

The overall accuracy of the quantitation method was 97 % for BAJ 2510.

The overall accuracy of the confirmation method was 97 % for spirodiclofen.

The overall accuracy of the confirmation method was 97 % for BAJ 2510.

Repeatability

As a measure for the precision of the method, the intra-laboratory repeatability for 5 replicates was determined in terms of RSD for all materials and at all fortification levels. The RSDs of the repeatability tests at each recovery set ranged from 1.6 to 14.1 % for BAJ 2740 and 0.8 to 11.7 % for BAJ 2510, respectively.

STUDY 2

reference	:	Bacher, R.; 2005, MO-05-005724	GLP statement	:	yes
type of study	:	Independent Laboratory Validation of Method 00919 for the Determination of Residues of spirodiclofen (BAJ 2740 and its enol metabolite BAJ 2510 in foodstuffs of animal origin by HPLC-MS/MS	guideline	:	EU guidelines SANCO/825/00
year of execution	:	2005	acceptability	:	yes
test substance	:	Spirodiclofen (BAJ 2740), purity 98.2 % (m/m) BAJ 2510, purity 99.9 % (m/m)			

Independent laboratory validation (ILV) data for the method for the determination of spirodiclofen and its metabolite BAJ 2510 in products of animal origin

The objectives of this study were to independently validate Bayer CropScience Method 00919 for the determination of residues of spirodiclofen (BAJ 2740) and its enol metabolite BAJ 2510 in foodstuffs of animal origin; and to demonstrate a LC/MS/MS confirmatory method.

The independent laboratory validation (ILV) was performed on whole milk and bovine meat. The confirmatory properties of the LC/MS/MS method were demonstrated by a second ion transition for both test items (second product ion / qualifier ion pair); 295 m/z for BAJ 2740 and at 231 m/z for BAJ 2510.

Recovery

A summary of the recovery results obtained is given in table B5.2e

Table B.5.2e Recoveries and Repeatability for spirodiclofen and BAJ 2510 (Quantitation and Confirmation Method)

Fortification Level (*)		Milk				Fortification Level (*)		Bovine Meat			
		BAJ 2740		BAJ 2510				BAJ 2740		BAJ 2510	
		411 -> 313 m/z	411 -> 295m/z	313 -> 213 m/z	313 -> 231 m/z			411 -> 313 m/z	411 -> 295m/z	313 -> 213 m/z	313 -> 231 m/z
0.005 mg/kg	Av.	86%	88%	92%	90%	0.010 mg/kg	Av.	100%	102%	101%	97%
	RSD	11%	13%	10%	12%		RSD	6%	4%	6%	4%
0.05 mg/kg (10xLOQ)	Av.	95%	96%	84%	84%	0.10 mg/kg	Av.	103%	105%	107%	103%
	RSD	7%	7%	10%	10%		RSD	3%	4%	4%	5%
Overall Av.		90%	92%	88%	87%	Overall Av.		101%	103%	103%	99%
Overall RSD		10%	11%	11%	11%	Overall RSD		5%	4%	5%	5%

Av.: Average. RSD: Relative standard deviation. Number of results included in calculation: 5 replicates per fortification level (except bovine meat 10xLOQ: 4 replicates).
(*): Fortification levels of enol metabolite BAJ 2510 given as equivalent of parent spirodiclofen (BAJ 2740)

Linearity

Comparison of the slopes of the calibration functions obtained with calibration solutions in solvent with those observed for the matrix-matched calibration solutions indicate that depending on the matrix both response enhancement and response suppression was observed for the analytes. The correlation coefficients (R) were always ≥ 0.994.

Specificity

The high selectivity (specificity) of the method resulted from the LC separation in combination with the very selective tandem mass spectrometric (MS/MS) detection. For confirmation of the detection of the analytes additional fragment ions are monitored.

No interfering signals in the duplicate blank control specimens were detected, resulting in a limit of detection (LOD) of 0.002 mg/kg for meat and 0.001 mg/kg for milk (20 % of LOQ).

Limit of Quantitation

The limit of quantification for the LC/MS/MS method, including the confirmatory ion transitions, was established at 0.01 mg/kg for meat and 0.005 mg/kg for milk (per analyte, concentrations of enol metabolite BAJ 2510 given as equivalent of parent spirodiclofen).

Accuracy

The single values of the recovery range from 73-109 % (milk and meat); overall accuracy of the quantitation method (n = 19) was 96 % for spirodiclofen. One single recovery value (172 %) was not used for calculation (Dixon outlier).

The single values of the recovery range from 77-109 % (milk and meat); overall accuracy of the quantitation method (n = 19) was 95 % for BAJ 2510. One single recovery value (175 %) was not used for calculation (Dixon outlier).

The single values of the recovery range from 71-110 % (milk and meat); overall accuracy of the confirmation method (n = 19) was 97 % for spirodiclofen. One single recovery value (177 %) was not used for calculation (Dixon outlier).

The single values of the recovery range from 75-107 % (milk and meat); overall accuracy of the confirmation method (n = 19) was 93 % for BAJ 2510. One single recovery value (182 %) was not used for calculation (Dixon outlier).

Repeatability

For both analytes examined by the original LC/MS/MS method and simultaneously by the confirmatory LC/MS/MS ion transition, for both foodstuffs of animal origin, and for each fortification level, the relative standard deviations (RSD) were ≤ 13 %.

B.5.6. References relied on

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No., GLP or GEP status,	Data Protection Claimed Y/N	Owner
IIA, 4.1.1	Kraemer, F.	2004a	Analytical Method AM001404MP1. Quantification of BAJ 2740 a.i.; HPLC-ISTD BCS, Report No. MO-04-006950 Date: 23-06-2004	Y	BAY
IIA, 4.1.3.1, 4.1.3.2, 4.1.3.3, 4.1.3.4	Kraemer, F.	2004b	Validation report for Method AM001404MP1. BCS, Report No. MO-04-006958 Date: 29-06-2004	Y	BAY
IIA, 4.1.3.1, 4.1.3.2, 4.1.3.3, 4.1.3.4	Odendahl A. and Zitzmann, W.	2008	Validation of HPLC-method AM011108MF1 - Determination of spirodiclofen and byproducts N,N-dimethylacetamide and BAJ 2740-enol in formulation – Spirodiclofen SC 240 (240 g/L), Generated by: Bayer AG Report N°: M-300489-01-1 Source: Bayer AG Date: 10-April 2008 Not GLP; Unpublished	Y	BAY
IIA, 4.2.1.1/11	Bacher, R.;,	2005	Independent Laboratory Validation of Method 00919 for the determination of residues of spirodiclofen (BAJ 2740 and its enol metabolite BAJ 2510 in foodstuffs of animal origin by HPLC-MS/MS Report N°: MO-05-005724 Source: Bayer AG Date: 02-03-2005 GLP; Unpublished	Y	Bayer AG

European Commission

SPIRODICLOFEN

ADDENDUM

VOLUME 4

ANNEX C

Rapporteur Member State: The Netherlands

~~**June 2005,**~~
~~**September 2006**~~
~~**April 2009**~~
June 2009

Draft Assessment Report and Proposed Decision of The Netherlands prepared in the context of the possible inclusion of spirodiclofen in Annex I of Council Directive 91/414/EEC