Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

	study initiation			
3.2.5	Number of animals per group	30/sex/group (F_0) 25/sex/group (F_1)		
3.2.6	Control animals	Yes		
3.3	**Administration/ Exposure**	Oral		
3.3.1	Duration of exposure before mating	Animals were fed the fenoxycarb-containing diet for a maturation period of 80 days for the F_0 generation and 100 days for the F_1 generation.		
		Oral		
3.3.2	Type	in food		
3.3.3	Concentration	food actual doses	200, 600, 1800 ppm see Table A6_8-2.1 below	X
3.3.4	Controls	Plain diet		
3.4	**Examinations**	In each generation, the parental animals were evaluated for treatment-related effects on survival, clinical condition, bodyweight gain, food consumption, reproductive performance and pathological changes. The offspring were evaluated for effects on viability, growth, clinical condition, physical and functional development and pathological changes.		
		4 RESULTS AND DISCUSSION		
4.1	**Effects**			
4.1.1	Parents	There were no treatment related mortalities in either generation of parents. In both generations, several females died during or shortly after parturition of the second litters. These deaths were attributed to the animals being past their peak of reproductive performance and to their unusually large litter sizes, and were considered incidental to treatment. There were no abnormalities of clinical condition in the parental animals of either generation that were considered to be related to treatment. During the pre-mating period, bodyweight gain of the F_0 parental animals were comparable with controls in the 200 and 600 ppm groups but marginally lower in the 1800 ppm group. For the remainder of the F_0 treatment period, body weight gain was comparable to controls in all groups. There was no effect of treatment at any dose level on bodyweight gain of the F_1 parental animals although the 1800 ppm animals were slightly lighter at the start of treatment. Food consumption during the pre-mating period was marginally lower than controls in the 1800 ppm females during the F_0 generation and in both sexes in the 1800 ppm group during the F_1 generation. There was no effect of treatment on the food consumption of the 200 and 600 ppm animals in either generation. Mating performance and fertility were unaffected by treatment at any of the dose levels tested at either the first or second mating in each generation. Slight reductions in the duration of gestation were observed in the 600 and 1800 ppm groups in the second mating of the first generation and in both matings in the second generation. **Pathology:** All 1800 ppm animals showed a statistically significant increase in both absolute and relative liver weight. A marginal increase was also noted in the 600 ppm females. Gonad weights were slightly increased in the 1800 ppm F_0 animals only, although there were no		X

		adverse effects on reproductive performance. At histopathological examination, treatment-related liver changes (hypertrophy/focal necrosis) were observed in the majority of the 1800 ppm F_0 or F_1 parental animals. Both the incidence and severity of these changes were increased compared with controls. There was no evidence of a treatment-related effect on any other tissue examined.	
4.1.2	Litters	There was no effect of treatment in either generation on the number of pups born, neonatal viability, clinical condition or necropsy findings of the offspring arising from either mating phase. A marginal reduction in the rate of weight gain was observed in both top dose (1800 ppm) litters in each of the F_0 and F_1 generations. Statistically significant differences in pup bodyweights were observed in all treatment doses. However, several arguments speak against a treatment-related effect on body weight gain, at least for the intermediate and low dose groups. Differences were not observed at all time points, and particularly on day 1, no significant weight differences were seen. There is virtually no dose dependency. Furthermore, there were no other effects observed on pup development, and pup bodyweight development is well within the historical control range. Therefore, the slight differences in pup body weight changes (at least at the lower doses), are considered not to be treatment-related and therefore not of toxicological relevance. The physical and functional development of the offspring of all treated animals was similar to controls in both litters of each generation. **Pathology:** In general, offspring in the 1800 ppm group showed increased liver weight, relative to bodyweight, although the effect was less distinct than that seen in the adults. There was no evidence of a treatment-related effect on the livers of the F_1b or F_2b pups selected for histopathological examination.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	X
5.1	**Materials and methods**	Technical grade OPP was administered via the diet at nominal doses of 0, 40, 140 and 490 mg/kg bw/day (actual doses were 35, 125 and 457 mg/kg bw/day) to CD Sprague-Dawley rats for two generations. Groups of 35 pairs of P parents and 27 males and 29 females (control) and 35 (low, middle and high dose groups) pairs of F_1 parents received OPP for 15 weeks and 10 weeks (post weaning), respectively, prior to mating. P animals were mated to produce F_{1a} and F_{1b} litters, and F_1 animals (F_{1b} pups randomly selected to be F_1 parents) were mated to produce F_{2a} and F_{2b} litters. Following weaning of the F_{1a} and F_{2a} pups, P and F_1 females were allowed to rest for six weeks and two weeks, respectively, before breeding for the subsequent generation. During the study, adult animals were evaluated for the effect of OPP on body weight, food consumption, clinical signs, oestrus cycling, fertility, gestation length and litter size. The offspring were evaluated for OPP-related effects on sex ratio, pup viability, body weight gain and clinical signs. Gross necropsy evaluations were performed on all adults and pups. Histopathological evaluation was performed on all adults and on gross lesions found on pups.	X
5.2	**Results and discussion**	There was no treatment-related increase in mortality and no compound-related clinical signs were observed. There was a statistically significant decrease in body weight gain in the high-dose group during the F_{1b}, F_{2a} and F_{2b} breedings. This decrease	X

		correlates with the lower body weights observed in P and F_1 adults and probably reflects consumption of the diet by the pups beginning during the second week of lactation. No effect on body weight was observed during the first week of lactation. This indicates no effect on early pup development. There was also an increased incidence of calculi in kidney and urinary bladder, renal haemorrhage and pyelonephritis in high-dose P males as well as an increased incidence of transitional cell hyperplasia / papillomatosis in the urinary bladder of high-dose P males and females. In the 125 mg/kg dose group increased incidence of calculi was found in the urinary bladder of male rats. Hyperplasia of the urothelium was found in P males and females. No treatment-related effects were observed for clinical signs, gestation and lactation body weight gain and reproductive parameters. There were also no treatment-related clinical signs in pups and no effects on pup viability. Gross and histopathological examinations of pups did not reveal any treatment-related lesions, and for adults no treatment-related lesions of the reproductive tract were observed. The reproductive NOEL was 40 mg/kg/day for both adults and resultant offspring. The overall NOEL for systemic organ toxicity in the adults, based on morphological changes, was 40 mg/kg/day. The physical and functional development of the offspring of all treated animals was similar to controls in both litters of each generation.	
5.3	**Conclusion**		X
5.3.1	LO(A)EL	see also Table A6_8_2-2	X
5.3.1.1	Parent males	LOAEL = 140 mg/kg bw/day	X
5.3.1.2	Parent females	LOAEL = 140 mg/kg bw/day	X
5.3.1.3	F1 males	LOAEL = 140 mg/kg bw/day	X
5.3.1.4	F1 females	LOAEL = 140 mg/kg bw/day	X
5.3.1.5	F2 males	LOAEL = not determined	X
5.3.1.6	F2 females	LOAEL = not determined	X
5.3.2	NO(A)EL		
5.3.2.1	Parent males	NOEL = 40 mg/kg bw/day	X
5.3.2.2	Parent females	NOEL = 40 mg/kg bw/day	X
5.3.2.3	F1 males	NOEL = 40 mg/kg bw/day	X
5.3.2.4	F1 females	NOEL = 40 mg/kg bw/day	X
5.3.2.5	F2 males	NOEL = not determined	X
5.3.2.6	F2 females	NOEL = not determined	X
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22/01/2007
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8-2.1. Ranges of dose received during the premating period (mg/kg bw/day)

Generation/Sex		Dietary concentration of fenoxycarb (ppm)		
		200	600	1800
F0 Parents:	Males	11.1 – 22.5	32.9 – 66.7	100.4 – 200.0
	Females	14.1 – 22.9	43.5 – 67.4	128.9 – 194.5
F1 Parents:	Males	10.2 – 21.1	30.5 – 63.6	92.2 – 191.5
	Females	12.4 – 21.0	37.7 – 64.4	111.7 – 185.7

Table A6_8-2.2. Table for reproductive toxicity study

Parameter		Generation	Controls		40 mg/kg		140 mg/kg		490 mg/kg		Dose-response	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Mortality	Incidence											
Found dead		P	0	2	1	0	0	1	3	0	–	–
		F_1	0	0	0	0	0	1	0	1	–	–
Mortality	Incidence											
Sacrificed prior to scheduled sacrifice		P	0	1	0	2	2	0	0	0	–	–
		F_1	0	0	0	0	2	0	2	0	–	–
Organ weights	%											
adult relative kidney weight [% of body weight]		P	0.806	0.825	0.802	0.839	0.798	0.859	0.873*	0.892	+	–
		F_1	0.748	0.886	0.788	0.844	0.791	0.868	0.834*	0.888	+	–
adult absolute kidney weight [g]		P	5.589	2.90	5.621	2.898	5.539	2.908	5.682	2.755	–	–
		F_1	4.923	3.075	5.249*	2.908	5,365*	2.955	4.901	2.795*	+	+
Body weight	g											
average weight		P	694.67	354.13	702.81	346.27	696.66	341.44	651.13*	310.03*	+	+
		F_1	660.89	350.73	668.92	345.17	679.67	341.44	590.04*	316.98*	+	+
Gross Pathology	Incidence											
Urinary bladder (calculi)		P	9/35	1/35	8/35	0/35	15/35	0/35	13/35	0/35	+	–
		F_1	3/27	1/29	2/35	0/35	4/35	0/35	7/35	0/33	+	–
Histopathology	Incidence											
Urinary bladder (transitional cell hyperplasia)		P	3/35	1/35	---	---	4/35	5/35	23/35*	9/35*	+	+
		F_1	1/27	6/29	1/35	4/35	4/35	1/34	15/35*	6/33	+	–

Reproductive Performance			Controls	40 mg/kg	140 mg/kg	490 mg/kg	Dose-response
Fertility Index Generation F_{1a}/GenerationF_{1b}		P	85/32	83/68*	85/64*	88/47	–
Fertility Index Generation F_{2a}/GenerationF_{2b}		F_1	89/83	89/77	82/70	94/88	–
Live birth index (# live pups day 0 / # of total pups per litter)	%	P	88	95	97*	100*	+
		F_1	100	99	98	96	–
Weight of viable pups Day 14	Mean [g]	F_{1a}	30	29	31	29	–
		F_{1b}	31	33	31	27*	+
		F_{2a}	32	33	32	30*	+
		F_{2b}	31	32	32	30	–
Weight of viable pups Day 21	Mean [g]	F_{1a}	49	47	48	45	–
		F_{1b}	49	50	48	40*	+
		F_{2a}	50	51	51	44*	+
		F_{2b}	51	50	51	45*	+

* statistically significant different from control $p \leq 0.05$;

RMS: This table should be deleted as it contains data from a different study, not data from the 2-generation study with fenoxycarb.

Evaluation by Rapporteur Member State, CA-Tables

CA-Table A6_8_2-1 Adult toxicity data

Parameter		Genera-tion	0 ppm		200 ppm		600 ppm		1800 ppm		Dose-response	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Body weight gain	g											
premating period before 1st litter		P	288.9	113.7	290.1	117.3	285.5	116.2	277.4	104.8	+	+
		F_1	337.7	140.3	333.8	136.8	348.8	137.5	323.9	122.7	+	+
Organ weights												
adult relative liver weight [% of body weight]		P	3.14	3.72	3.15	3.88	3.27	4.04*	3.74*	4.83*	+	+
		F_1	2.99	4.01	2.95	4.14	3.09	4.75*	3.45*	5.59*	+	+
adult absolute liver weight [g]		P	17.9	12.4	18.3	12.9	18.5	13.2	21.1*	14.9*	+	+
		F_1	17.7	14.2	17.2	14.3	18.7	16.2*	19.7*	18.2*	+	+
Histopathology	Incidence											
hepatocyte hypertrophy		P	0	0	-	-	-	-	20	13	+	+
		F_1	0	0	-	-	-	-	24	14	+	+
focal necrosis (liver)		P	2	0	-	-	-	-	5	1	+	–
		F_1	1	1	-	-	-	-	14	0	+	–
* statistically significant different from control $p \leq 0.05$												

CA-Table A6_8_2-2 Reproduction and litter data

Reproductive Performance		0 ppm	200 ppm	600 ppm	1800 ppm	Dose-response
Pregnant females	P/ F_{1a}	29/30	30/30	29/30	30/30	–
	P/ F_{1b}	28/30	27/29	28/30	29/30	–
	F_1/ F_{2a}	21/25	23/25	23/25	24/25	–
	F_1/ F_{2b}	20/25	21/25	23/25	24/25	–
Live litters Day 21 pn	F_{1a}	28	28	28	30	–
	F_{1b}	23	21	27	25	–
	F_{2a}	19	21	20	23	–
	F_{2b}	15	19	22	23	–
Litters with > 1 stillborn pup	F_{1a}	3	3	2	1	–
	F_{1b}	3	3	2	5	–
	F_{2a}	5	5	5	1	–
	F_{2b}	6	1	3	3	–
Litter size Day 1 pn	F_{1a}	14.4	14.4	14.2	14.1	–
	F_{1b}	13.5	14.1	13.9	13.4	–
	F_{2a}	13.1	13.8	14.0	13.5	–
	F_{2b}	12.9	13.9	13.9	14.0	–
Pup weight Day 1 pn Mean [g]	F_{1a}	5.7	5.6	5.6	5.6	–
	F_{1b}	5.9	5.8	5.6	5.8	–
	F_{2a}	6.1	5.6	5.6	5.8	–
	F_{2b}	6.3	6.0	5.8	6.0	–
Pup weight Day 21 pn Mean [g]	F_{1a}	49	45*	46*	44*	–
	F_{1b}	49	48	47	44*	+
	F_{2a}	48	44	44*	42*	+
	F_{2b}	50	45*	44*	40*	+
* statistically significant different from control $p \leq 0.05$						

CA-Table A6_8_2-3 Duration of pregnancy

Pregnancy	control		200 ppm		600 ppm		1800 ppm	
	21 days	22 days	21 days	22 days	21 days	22 days	21 days	22 days
F0 → F1a	14	15	18	11	20	9	21	9
F0 → F1b	8	15	16	9*	21	7*	21	6*
F1 → F2a	6	15	9	14	13	10	17	6*
F1 → F2b	4	12	11	6*	15	5*	16	6*
* statistically significant different from control $p \leq 0.05$; Permutation test, one-tailed								

CA-Table A6_8_2-4 Neonates with haemorrhage or haematoma[1]

Generation	control		200 ppm		600 ppm		1800 ppm	
	Litters	Pups	Litters	Pups	Litters	Pups	Litters	Pups
F1a	4	7	8	9	3	3	9	10
F1b	2	6	10	17	12	19	12	19
F2a	2	2	5	9	5	6	7	10
F2b	3	13	9	15	10	14	10	14

[1] observational data, apparently no systematic evaluation of this endpoint in neonates

Section A6.9 Neurotoxicity Studies

Annex Point IIIA VI.1 6.9 Investigation of cholinesterase activity in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1982), Effect Of Ro 13 5223/000 (IGR) On Plasma Cholinesterase In Rats. [redacted], Report No. B-46202, 10 August 1982 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Syngenta	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	This was an investigative study with no applicable guidelines.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Fenoxycarb (Ro 13-5223/000)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Purity	[redacted]	
3.1.2.2	Stability	not reported	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	[redacted] albino (SPF)	
3.2.3	Source	not reported	X
3.2.4	Sex	♀	
3.2.5	Age/weight at study initiation	not reported	X
3.2.6	Number of animals per group	10	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	24 h	
3.3.2	Type	Gavage	
3.3.3	Concentration	5000 mg fenoxycarb/kg bw	
3.3.4	Vehicle	Sodium-carboxymethylcellulose (5 g), Tween 80 (4 mL), benzyl alcohol (5 mL), NaCl (9 g), made up to 1000 mL with distilled water	

Section A6.9		Neurotoxicity Studies	
Annex Point IIIA VI.1		6.9 Investigation of cholinesterase activity in rats	
3.3.5	Control	Neg. control: Vehicle Pos. control: 500 mg/kg of carbaryl was orally given to a third group of 10 rats and 80 mg/kg of chlorpyrifos was orally administered to a fourth group of 10 rats.	
3.4	**Examinations**	General behaviour, plasma cholinesterase, body weights	X
3.5	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	One rat in the carbaryl group was found dead, 6 hours after dosing. All other rats in this group were heavily sedated, had foam around the mouth and uncontrolled urination. Due to the poor state of the animals, blood was taken from the remaining 9 females in this group and the animals were then killed for humane reasons. All animals in all other groups appeared normal 6 and 24 hours after dosing.	
4.2	**Cholinesterase activity**	No inhibitory effect on rat plasma cholinesterase was observed 24 hours after a single dose of 5000 mg/kg fenoxycarb, administered orally by gavage. The plasma cholinesterase levels of the rats dosed with fenoxycarb were similar to those of the vehicle control group. The rats dosed with 500 mg/kg of carbaryl revealed a 78% inhibition and rats dosed with 80 mg/kg of chlorpyrifos showed approximately 94% inhibition of plasma cholinesterase activity, when compared with the plasma cholinesterase levels of the rats from the vehicle control group (see Table A6_9-1).	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A group of 10 female, [redacted], albino, rats (outbred stock, SPF-quality), were given a single oral dose of 5000 mg/kg fenoxycarb by gavage. Another group of 10 rats received an equivalent volume of the vehicle (sodium-carboxymethylcellulose (5 g), Tween 80 (4 mL), benzyl alcohol (5 mL), NaCl (9 g), made up to 1000 mL with distilled water) alone, as a negative control. Furthermore, 500 mg/kg of technical grade carbaryl was orally given to a third group of 10 rats and 80 mg/kg of technical grade chlorpyrifos was orally administered to a fourth group of 10 rats, both serving as positive controls. Bodyweights were recorded prior to dosing. The general behaviour of the animals was recorded 6 and 24 hours after administration of the compounds. Blood samples were taken 24 hours after dosing, except in the carbaryl group where blood was taken 6 hours after dosing because of the poor state of health of the animals. Plasma was separated from the blood cells by centrifugation and the cholinesterase activity was determined immediately for all samples.	
5.2	**Results and discussion**	No inhibitory effect on plasma cholinesterase was observed in rats receiving 5000 mg/kg fenoxycarb and in rats in the vehicle control group. In contrast, rats in the carbaryl group revealed a 78% inhibition and rats in the chlorpyrifos group showed roughly a 94% inhibition of plasma cholinesterase activities, when compared with the plasma cholinesterase levels of the vehicle controls.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Section A6.9 Neurotoxicity Studies

Annex Point IIIA VI.1 6.9 Investigation of cholinesterase activity in rats

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/09
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

TableA6_9-1: Cholinesterase (ChE) inhibition

Group	n	ChE activity (µmol/min/L)	% of vehicle control
Vehicle control	10	394 ± 104	100
Fenoxycarb	10	397 ± 88.3	101
Carbaryl	9	86.6 ± 30.8#	22.0
Chlorpyrifos	10	25.1 ± 6.09#	6.37

n =number of animals # = statistically significantly different from vehicle control

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of hepatic enzyme induction in mice

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1996a), CGA 114597 tech.(Fenoxycarb) - Effects On Biochemical Liver Parameters Following Dietary Administration To Male And Female Mice. [redacted], Report No. CB 95/36, 15 January 1996 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	Syngenta
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This was an investigative study with no applicable guidelines.
2.2	**GLP**	No
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Fenoxycarb (CGA 114597) technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Purity	[redacted]
3.1.2.2	Stability	Analysis of the diets showed that the achieved concentrations, stability and homogeneity of fenoxycarb in the diet were satisfactory.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Tif:MAGf(SPF)
3.2.3	Sex	♂+♀
3.2.4	Age/weight at study initiation	Young adult
3.2.5	Number of animals per group	20
3.2.6	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	28 days (recovery group only)
3.3.2	Type	Dietary
3.3.3	Concentration	0, 50, 500 and 2000 ppm ≅ 0, 10.1, 92.9 and 365.0 mg/kg bw/day for ♂ 0, 10.0, 91.7 and 361.6 mg/kg bw/day for ♀
3.3.4	Vehicle	None
3.3.5	Control	Plain diet

Section A6.10	Mechanistic Studies
Annex Point IIIA VI.7	6.10 Investigation of hepatic enzyme induction in mice
3.3.6 Duration of exposure	14 days
3.4 Examinations	Body weights, food consumption, gross pathology, organ weights. Hepatic biochemical parameters: protein content of 100*g* supernatant, microsomal and cytosolic fractions, microsomal cytochrome P450 content, microsomal 7-ethoxyresorufin- and 7-pentoxyresorufin-O-dealkylase activities, cytosolic glutathione S-transferase activity, cyanide-insensitive peroxisomal β-oxidation and microsomal lauric acid 11- and 12-hydroxylation.
3.5 Further remarks	None
	4 RESULTS AND DISCUSSION
4.1 General observations	There were neither mortalities nor any clinical signs of toxicity detected throughout either the treatment or recovery period. There was no effect of fenoxycarb administration on either bodyweight or food consumption.
4.2 Gross pathology, organ weights	There were no macroscopic changes observed at examination *post mortem*. There were no treatment-related effects on the carcass weight. The absolute and relative liver weights were dose-dependently increased at 500 and 2000 ppm in males and females and were very slightly, but statistically, increased in females at 50 ppm (see Table A6_10-1). The increased liver weights reverted to levels similar to those of the controls after a 28-day recovery period. There was no treatment-related effect on lung weights.
4.3 Biochemical Liver Parameters:	The major treatment-related biochemical alterations were an increase in cytochrome P450 content up to 166% and 147% of control at the highest dose in male and female animals, respectively. This was paralleled by a strong increase in protein cross reacting with the monoclonal antibody clo4 specific for rat CYP4A isoenzymes, as well as strong induction of lauric acid 12-hydroxylation up to 753% and 1254% of control at 2000 ppm for male and female animals, respectively. The peroxisomal fatty acid ß-oxidation was moderately induced at 500 and 2000 ppm and attained 203% and 243% of control at the highest dose for male and female animals, respectively. Minor biochemical alterations comprised a slight to moderate induction of lauric acid 11-hydroxylase activity up to 245 and 226% of control as well as slight increases in ethoxyresorufin O-deethylase activity up to 224% and 186% of control and pentoxyresorufin O-depentylase activity up to 463% and 177% of control at the highest dose for male and female animals, respectively. In addition, slightly increased microsomal protein contents (114-118% of control) were observed in males at 500 and 2000 ppm. Treatment had no effect on protein contents of 100*g* supernatant and cytosolic liver fractions, glutathione S-transferase and protein detected with two monoclonal antibodies specific for rat CYP1A and CYP3A isoenzymes. All treatment related effects were reversible after the 28-day recovery period (see TableA6_10-2).

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of hepatic enzyme induction in mice

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Groups of 20 young adult male and female Tif:MAGf(SPF) mice were treated for 14 consecutive days with fenoxycarb technical at dietary concentrations of 0, 50, 500 and 2000 ppm, corresponding to mean daily doses of 0, 10.1, 92.9 and 365.0 mg/kg bodyweight for males and 0, 10.0, 91.7 and 361.6 mg/kg for females. The male mice were housed individually and the females were housed 5 per cage.

In order to test the reversibility of possible treatment-related effects, groups of 20 animals per sex received 0 or 2000 ppm for 14 days followed by a 28-day recovery period.

Individual bodyweights and food consumption were recorded daily during the 14-day treatment period and twice per week during the recovery period.

At the end of the study the animals were killed and livers and lungs removed and weighed. The lungs of 10 mice per group were pooled. Frozen liver and lung samples were stored at -80°C.

The following biochemical parameters were determined from the livers of 2 females and 8 males per group: protein content per 100*g* supernatant, microsomal and cytosolic fractions, microsomal cytochrome P450 content, microsomal 7-ethoxyresorufin- and 7-pentoxyresorufin-O-dealkylase activities, cytosolic glutathione S-transferase activity, cyanide-insensitive peroxisomal β-oxidation and microsomal lauric acid 11- and 12-hydroxylation.

5.2 Results and discussion

The liver enzyme activity profile in treated male and female mice, i.e. prominent increase in lauric acid 12-hydroxylation and moderate increase in fatty acid β-oxidation together with a strong increase of protein detected with the monoclonal antibody clo4 specific for rat liver CYP4A isoenzymes, indicated that fenoxycarb is a strong inducer of hepatic xenobiotic metabolising enzymes in the mouse and can be classified as a peroxisome proliferator type inducer.

The lowest dose of 50 ppm is a minimal effect level. Hydroxylation of lauric acid at the 12-position was slightly induced in the female animals and at the 11-position in both sexes. A minimal increase of the ethoxyresorufin O-de-ethylase activity at this dose in male animals was neither followed by a substantial further increase at the two higher doses nor paralleled by an increase of CYP1A proteins. The minimal increases of the absolute and relative liver weights in female animals at the lowest dose were in the range of 10% above control and are regarded as borderline. In a cell proliferation study in male mice, dietary administration of fenoxycarb at 500 ppm caused an increase of the relative and absolute liver weight after 14 days treatment. However, after treatment for 42 days at the same dose level, this increase was reduced and no more statistically significant, indicating a certain adaptation ([redacted] *1996*, summarised separately). Correspondingly, the minimal effects at the lowest dose of 50 ppm in the female animals as observed in this study might be transient.

5.3 Conclusion

5.3.1 Reliability 0 - Supplementary data

5.3.2 Deficiencies None

X

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of hepatic enzyme induction in mice

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/08
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

TableA6_10-1: Intergroup comparison of liver weights (absolute and relative to carcass weight)

	Dietary concentration of fenoxycarb (ppm)											
	Males						Females					
					Recovery						Recovery	
	0	50	500	2000	0	2000	0	50	500	2000	0	2000
Absolute (g)	1.80	1.80	2.09***	2.42***	1.88	1.88	1.42	1.57*	1.73***	1.88***	1.59	1.71
Relative (%)	5.60	5.75	6.33***	7.23***	5.20	5.45	5.43	5.86**	6.39***	7.22**	5.23	5.42

** Statistically significant difference from control group mean, p<0.01 (Dunnett's test, 2-sided)
*** Statistically significant difference from control group mean, p<0.001 (Dunnett's test, 2-sided)

TableA6_10-2: Intergroup comparison of selected biochemical liver parameters

	Dietary concentration of fenoxycarb (ppm)											
	Males						Females					
					Recovery						Recovery	
	0	50	500	2000	0	2000	0	50	500	2000	0	2000
Protein content of microsomal fraction [mg/g liver]	19.5	18.8	23.0*	22.2	20.3	17.0	20.8	19.3	22.1	21.9	17.8	17.9
Microsomal cytochrome P450 content [nmol/g liver]	15.1	17.7	23.1***	25.1***	13.7	12.8	16.4	15.2	20.7*	24.0***	11.0	10.4
Microsomal EROD [nmol/min/g liver]	3.1	4.5**	6.1***	6.9***	3.5	3.8	5.0	4.5	8.2***	9.3***	3.9	3.6
Microsomal PROD [nmol/min/g liver]	0.44	0.57	1.22***	2.02***	0.53	0.63	1.98	1.73	2.80**	3.50***	2.05	1.98
LA-11-OH activity [nmol/min/g liver]	18.6	23.9*	36.6***	45.6***	21.0	21.9	30.9	42.1*	58.8***	69.8***	31.1	31.4
LA-12-OH activity [nmol/min/g liver]	17.7	23.3	73.2***	133.3***	20.9	35.2	23.9	40.0**	153.6***	299.8***	25.1	23.2
FAO activity [nmol/min/g liver]	1002	1097	1473***	2036***	907	948	1124	1102	1524***	2736***	1150	1103
Monoclonal antibody d15[a)]	1595	1616	1684	1261	1759	1817	906	451	527	367	627	466
Monoclonal antibody p6[a)]	0/1302	0/1973	0/1748	0/2393	0/1874	0/1679	718/204	622/384	587/337	839/187	580/379	395/525
Monoclonal antibody clo4[a)]	450	415	2891	2333	845	1121	564	477	2993	4207	845	304

* Statistically significant difference from control group mean, p<0.05 (Dunnett's test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Dunnett's test, 2-sided)
*** Statistically significant difference from control group mean, p<0.001 (Dunnett's test, 2-sided)
EROD ethoxyresorufin O-deethylase
PROD pentoxyresorufin O-depentylase
LA-11-OH lauric acid 11-hydroxylase
LA-12-OH lauric acid 12-hydroxylase
FAO fatty acid ß-oxidation
a) Monoclonal antibody assay: densitometric scanning of bands on immunoblot. No statistics

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of enzyme induction in murine lung

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1 REFERENCE

1.1	**Reference**	[redacted] (1996b), CGA 114597 tech. (Fenoxycarb) - Effects On Biochemical Lung Parameters Following Dietary Administration To Male And Female Mice. [redacted], Report No. CB 95/46, 7 June 1996 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Syngenta
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This was an investigative study with no applicable guidelines.
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Fenoxycarb (CGA 114597) technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Purity	[redacted]
3.1.2.2	Stability	Three sub samples of each concentration of the test substance in the diet were analysed for content and homogeneity. The actual test substance concentrations in the diet were 55.7, 527 and 2142 ppm which correspond to 111%, 105% and 107% of the respective nominal values, and thus met the usual quality standard which allows deviations of 25% from the nominal value. For each concentration, the deviation of individual values was within ± 15% of the mean value and therefore the diet batches were considered homogenous. In a previous study ([redacted], 1993), the test substance was shown to be stable in pelleted diet at concentrations of 10, 100, 1000 and 20000 ppm for at least 5 weeks when stored at room temperature.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Tif:MAGf(SPF)
3.2.3	Sex	♂+♀
3.2.4	Age/weight at study initiation	Young adult
3.2.5	Number of animals per group	20
3.2.6	Control animals	Yes

Section A6.10		Mechanistic Studies	
Annex Point IIIA VI.7		6.10 Investigation of enzyme induction in murine lung	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	28 day (recovery group only)	
3.3.2	Type	Dietary	
3.3.3	Concentration	0, 50, 500 and 2000 ppm ≅ 0, 10.1, 92.9 and 365.0 mg/kg bw/day for ♂ 0, 10.0, 91.7 and 361.6 mg/kg bw/day for ♀	
3.3.4	Vehicle	None	
3.3.5	Control	Plain diet	
3.3.6	Duration of exposure	14 days	
3.4	**Examinations**	Body weights, food consumption, gross pathology, organ weights. Lung biochemical parameters: protein content of 100*g* supernatant, microsomal and cytosolic fraction, microsomal 7-ethoxy- and 7-pentoxyresorufin O-dealkylase, microsomal lauric acid 11- and 12-hydroxylase, peroxisomal fatty acid β-oxidation, cytosolic glutathione S-transferase, abundance of microsomal proteins cross reacting with monoclonal antibodies specific for rat cytochrome P450 isoenzymes of families CYP1A, CYP3A and CYP4A.	X
3.5	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**General observations**	No treatment-related clinical signs were recorded during the treatment or recovery periods. There was no effect on bodyweight development or food consumption.	
4.2	**Gross pathology, organ weights**	No macroscopic changes were observed at necropsy at any test substance concentration tested. No effects of treatment were seen on the absolute or relative lung weights at any dose level.y period. There was no treatment-related effect on lung weights.	
4.3	**Biochemical parameters:**	**Protein content of subcellular fractions:** Treatment had no effect on the protein content of the 100*g* supernatant, the microsomal or the cytosolic fraction. **Microsomal 7-ethoxyresorufin O-de-ethylase (EROD):** In both sexes treatment was without an inducing effect on lung EROD activity. Due to the considerable variation between the pools of the same group, the slight variations between the treatment groups are regarded as incidental and not biologically relevant. **Microsomal 7-pentoxyresoufin O-depentylase (PROD):** In both sexes, the differences in lung PROD activity between treated and the corresponding control groups were marginal and regarded as incidental and not biologically significant. **Microsomal lauric acid 11- and 12-hydroxylase:** Lung lauric acid 11- and 12-hydroxylation activities were not affected by treatment in either male or female animals. **Peroxisomal fatty acid ß-oxidation:** Treatment had no effect on lung peroxisomal fatty acid ß-oxidation in male and female animals. **Cytosolic glutathione S-transferase:** Treatment had no effect on the lung cytosolic glutathione S-transferase. **Cytochrome P450 isoenzymes analysed by immunoblot:**	

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of enzyme induction in murine lung

The monoclonal antibody d15 was raised against and is specific for rat liver isoenzymes CYP1A1 and CYP1A2. In lung microsomes of control and treated mice of both sexes, this antibody did not recognise any protein.

The monoclonal antibody p6 was raised against and is specific for rat liver isoenzymes CYP3A1 and CYP3A2. In lung microsomes of control and treated mice of both sexes, this antibody did not recognise any protein.

The monoclonal antibody clo4 was raised against and is specific for peroxisome proliferator-inducible rat liver CYP4A isoenzymes. In lung microsomes from untreated male or female mice it recognised one band that might be attributable to isoenzyme CYP4B1, a major component of the P450 monoxygenase system in mouse Clara cells. Treatment in this study had no effect on the intensity of this protein band.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Groups of 20 young adult male and female Tif:MAGf(SPF) mice were treated for 14 consecutive days with fenoxycarb at dietary concentrations of 0, 50, 500 or 2000 ppm. In order to test for the reversibility of possible treatment-related effects, groups of 20 animals per sex received 0 or 2000 ppm for 14 days followed by a 28 day recovery period.

At scheduled termination, the animals were killed and the lungs quickly removed and weighed. The lungs from 10 mice of each group were pooled and immediately chopped and then frozen in liquid nitrogen. The frozen samples were stored at -80°C until processed (subcellular fractionation).

Biochemical lung parameters were investigated in two pools of ten animals each for all dose groups and both sexes. The following parameters were determined in subcellular lung fractions: protein content of 100*g* supernatant, microsomal and cytosolic fraction, microsomal 7-ethoxy- and 7-pentoxyresorufin O-dealkylase, microsomal lauric acid 11- and 12-hydroxylase, peroxisomal fatty acid β-oxidation, cytosolic glutathione S-transferase as well as abundance of microsomal proteins cross reacting with monoclonal antibodies specific for rat cytochrome P450 isoenzymes of families CYP1A, CYP3A and CYP4A.

5.2 Results and discussion

Treatment of male and female mice for 14 days with fenoxycarb at 50, 500 and 2000 ppm was without a macropathological effect on the lungs or on the absolute and relative lung weight. The lack of any remarkable effect on biochemical lung parameters, i.e. protein content of subcellular fractions, microsomal 7-ethoxyresorufin O-deethylase and 7-pentoxy-resorufin O-dependentylase, microsomal lauric acid 11- and 12-hydroxylase, peroxisomal fatty acid β-oxidation, cytosolic glutathione S-transferase and microsomal protein detected with monoclonal antibodies specific for rat liver cytochrome P450 isoenzymes of gene families CYP1A, CYP3A and CYP4A, indicate that fenoxycarb has no inductive properties on pulmonary xenobiotic metabolising enzymes in the mouse.

X

5.3 Conclusion

5.3.1 Reliability 0 - Supplementary data

5.3.2 Deficiencies None

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of enzyme induction in murine lung

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/04/04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of DNA replication in murine lung and liver

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1 REFERENCE

1.1	**Reference**	[redacted] (1996), CGA 114597 tech. - Assessment Of Replicative DNA Synthesis In The Lung And Liver Of Male Mice Treated For 7, 14 And 42 Days. Investigation Of The Reversibility In A 42-Day Treatment/28-Day Recovery Experiment. [redacted], Report No. CB 95/03, 25 May 1996 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Syngenta
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This was an investigative study with no applicable guidelines.
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Fenoxycarb (CGA 114597) technical
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Purity	[redacted]
3.1.2.2	Stability	The test substance was demonstrated to be stable in the dosing vehicle in a previous study, ([redacted]1995) for the period of use during the study. The test substance was shown to be stable in pelleted diet at a concentration of 5000 ppm for 7 weeks when stored at room temperature and thus covered the period of use in this study. Three sub samples of each concentration were analysed for content and homogeneity of the test substance. The test substance concentrations in the diets were found to be 49.5, 452 and 1747 ppm, which corresponds to 99.0, 90.4 and 87.4% of the nominal value and thus met the usual quality standards, which allow deviations of ±25% from the nominal values. For each concentration, the deviation of the individual values was within 15% of the mean value and therefore the diet batches were considered to be homogenous.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Tif:MAGf(SPF)
3.2.3	Sex	♂
3.2.4	Age/weight at study initiation	Young adult
3.2.5	Number of animals per group	5
3.2.6	Control animals	Yes

Section A6.10	Mechanistic Studies
Annex Point IIIA VI.7	6.10 Investigation of DNA replication in murine lung and liver

3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	28 day (recovery group only)
3.3.2	Type	Dietary
3.3.3	Concentration	7-d treatment group: 0 and 2000 ppm ≅ 0 and 302.9 mg/kg bw/day 14-d treatment group: 0, 50, 500 and 2000 ppm ≅ 0, 8.5, 75.0 and 297.5 mg/kg bw/day 42-d treatment group: 0, 50, 500 and 2000 ppm ≅ 0, 7.2, 68.7 and 259.5 mg/kg bw/day 42-d treatment/28-d recovery group: 0 and 2000 ppm ≅ 0 and 271.1 mg/kg bw/day
3.3.4	Vehicle	None
3.3.5	Control	Plain diet Two groups of 5 animals each, treated with either a single intraperitoneal dose of 400 mg/kg bw butylated hydroxytoluene (BHT) in corn oil or with the vehicle alone seven days before sacrifice, served as a positive control for pulmonary cell proliferation throughout this experiment.
3.3.6	Duration of exposure	7, 14 or 42 days
3.4	**Examinations**	Body weights, food consumption, gross pathology, organ weights, immunohistochemical analysis of DNA replication in lung and liver sections
3.5	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1	**General observations**	There were no deaths during this study. No in-life clinical observations were recorded for the animals treated with fenoxycarb. One animal in the 400 mg/kg BHT group showed piloerection on day 5 of the study. There was no effect of treatment with either fenoxycarb or BHT on body weight development or food consumption.
4.2	**Gross pathology, organ weights, histopathology**	There were no treatment-related macroscopic changes observed at necropsy. The mean liver weight was increased by 23%, 36% and 25% in the 2000 ppm groups after 7, 14 and 42 days, respectively. Treatment with 500 ppm fenoxycarb for 14 days resulted in a 36% increase. Similar carcass weights were seen across all groups and the resultant liver to bodyweight ratios were also increased by 17%, 31% and 26% in the 2000 ppm animals treated for 7, 14 and 42 days, respectively and 24% in the 500 ppm animals treated for 14 days. There was no effect on mean liver weight after treatment with 50 ppm fenoxycarb. The effect on the liver weight at the highest dose of 2000 ppm was reversible as demonstrated by the liver weights of the 28 day recovery animals, where the weights were similar to the respective controls. Single intraperitoneal injections of 400 mg/kg bw BHT did not produce any effect on the mean or relative liver weights of these animals. Histopathological examination of the liver showed an increased incidence and severity of hepatocellular hypertrophy associated with a reduced glycogen content in animals treated with 2000 ppm fenoxycarb for 7, 14 or 42 days, respectively. The effect was reversible as there was no indication of hypertrophy in animals treated for 42 days with 2000

ppm fenoxycarb and allowed to recover for 28 days (see Table A6_10-1).

All other microscopic findings in the liver were considered to be incidental to treatment. A single intraperitoneal injection of 400 mg/kg bw BHT did not result in any treatment-related histopathological changes in the liver.

Histopathological examination of the lung showed no findings related to treatment with fenoxycarb. Intraperitoneal injection of 400 mg/kg bw BHT, when examined 7 days later, resulted in various lesions of the alveolar compartment of the lung, indicative of a damaging effect. There was an increased cellularity, due to infiltration with leucocytes and macrophages and, occasionally, necrosis and accumulation of foam cells were observed.

4.3 DNA replication

Appropriate staining for PCNA was checked with the testis sections of the control animal where the prominent PCNA staining of spermatogonia confirmed the suitability of the methodology.

There was a slight increase in the mean hepatocellular PCNA labelling index after 7 days of treatment with 2000 ppm fenoxycarb and after 14 days of treatment with 500 or 2000 ppm fenoxycarb, that was statistically significant at 500 ppm only. Treatment for 42 days at 2000 ppm did not produce any difference in the mean labelling index between control and treated animals, indicating the transient nature of the effect. Likewise, there was no difference in the mean labelling index between treated and control animals of the 42–day treatment/28-day recovery group. A single intraperitoneal injection of 400 mg/kg bw BHT resulted in a slight increase in the mean hepatocellular labelling index (see Table A6_10-2).

There was no indication of an effect of fenoxycarb or BHT on the PCNA labelling index of bronchiolar epithelial cells.

Except for those animals treated with 50 ppm fenoxycarb for 42 days, there were no statistically significant increases in mean PCNA labelling index of alveolar cells. There was no indication of a dose related effect in the 500 and 2000 ppm groups and therefore the increase at 50 ppm was considered incidental, excluding induction of pulmonary cell proliferation by fenoxycarb. In contrast, treatment with the reference compound BHT, known to induce proliferation of alveolar parenchymal cells, resulted in a clear-cut and strong 4.7-fold increase of the fraction of DNA replicating cells (see Table A6_10-3).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Groups of five young adult male Tif:MAGF (SPF) mice were treated with fenoxycarb for 7 consecutive days at dietary concentrations of 0 or 2000 ppm, or for either 14 days or 42 days at dietary concentrations of 0, 50, 500 or 2000 ppm. In order to test for the reversibility of possible treatment-related effects, groups of 5 animals received 0 or 2000 ppm for 42 days followed by a 28-day recovery period.

Two groups of 5 animals each, treated with either a single intraperitoneal dose of 400 mg/kg bw butylated hydroxytoluene (BHT) in corn oil or with the vehicle alone seven days before sacrifice, served as a positive control for pulmonary cell proliferation throughout this experiment.

Each animal was checked for viability and clinical signs at least once daily during the treatment and recovery period. Individual body weights

Section A6.10	Mechanistic Studies
Annex Point IIIA VI.7	6.10 Investigation of DNA replication in murine lung and liver

		were recorded and daily food consumption was determined throughout the study. At the end of the scheduled period, the animals were killed and a detailed necropsy performed. The lung and liver of all animals and the testes from a control animal were removed. The liver was weighed and preserved in 4% neutral buffered formalin. Prior to immersion fixation, the lung was endotracheally instilled with fixative to optimise tissue preservation. Formalin fixed liver and lung tissue was used for histopathological examination of haematoxylin/eosin stained sections and for immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) as an indicator of DNA-replicating cells. The testes removed from one control animal were preserved in 4% neutral buffered formalin and served as a positive control in PCNA-immunohistochemistry.
5.2	**Results and discussion**	Subchronic treatment of male mice with 500 and 2000 ppm fenoxycarb resulted in hepatomegaly, which correlated with a reversible hepatocellular hypertrophy at 2000 ppm and a transiently increased proliferation of hepatocytes at 500 and 2000 ppm. There was no effect at any dose and study duration on lung morphology and pulmonary cell proliferation. The 50 ppm dose level, corresponding to approximately 8 mg/kg bw/day, represents the no observable effect level (NOEL).
5.3	**Conclusion**	
5.3.1	Reliability	0 - Supplementary data
5.3.2	Deficiencies	None

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Annex Point IIIA VI.7 6.10 Investigation of DNA replication in murine lung and liver

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/08
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1: Intergroup comparison of histopathology of the liver – incidences (n=5) of selected findings

Group/Finding	Dietary fenoxycarb concentration (ppm)			
	0	**50**	**500**	**2000**
7-day treatment				
Hypertrophy hepatocellular	0	-	-	3
14-day treatment				
Hypertrophy hepatocellular	0	1	0	4
42-day treatment				
Hypertrophy hepatocellular	0	0	1	3
42-day treatment/28 day recovery				
Hypertrophy hepatocellular	0	-	-	0
Intraperitoneal injection BHT	**0 (control)**		**400 mg/kg bw**	
Hypertrophy hepatocellular	0		0	

Table A6_10-2: PCNA-analysis of the liver

Labelling index of hepatocytes in percent – mean (number of animals examined)					
Group	**Control**	**Fenoxycarb 50 ppm**	**Fenoxycarb 500 ppm**	**Fenoxycarb 2000 ppm**	**BHT 400 mg/kg bw**
7 days treatment	0.59 (4)	-	-	0.92 (5) p=0.055	-
14 days treatment	0.41 (5)	0.50 (5) p=0.111	0.74 (5) p=0.016	0.72 (5) p=0.075	-
42 days treatment	0.20 (5)	n.r.	n.r.	0.22 (5) p=0.274	-
42 days treatment / 28 days recovery	0.35 (5)	-	-	0.35 (5) p=0.579	-
BHT, single intraperitoneal injection + 7 days	0.59 (5)	-	-	-	0.85 (4) p=0.278

p = p-value of one sided Mann-Whitney –Test
n.r. = examination not requested.

Table A6_10-3: PCNA-analysis of the lung, alveolar compartment

Cells of the alveolar compartment					
	Labelling index (%) – mean (number of animals examined)				
Group	**Control**	**Fenoxycarb 50 ppm**	**Fenoxycarb 500 ppm**	**Fenoxycarb 2000 ppm**	**BHT 400 mg/kg bw**
7 days treatment	0.92 (5)	-	-	0.33 (5) p= 1.0	-
14 days treatment	0.53 (5)	0.77 (5) p=0.345	0.74 (5) p=0.275	0.86 (5) p=0.155	-
42 days treatment	0.49 (5)	0.97 (5) p= 0.008	0.79 (5) p= 0.075	0.61 (5) p= 0.210	-
42 days treatment / 28 days recovery	0.68 (5)	-	-	0.84 (5) p= 0.345	-
BHT, single intraperitoneal injection + 7 days	0.73 (5)	-	-	-	3.45 (5) p= 0.004

p = p-value of one sided Mann-Whitney -Test

4Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver and lung

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1 REFERENCE

1.1 Reference [redacted] (1997), CGA 114597 (Fenoxycarb) - *In Vitro* Metabolism By Liver And Lung Of Mouse, Rat, Marmoset And Man. [redacted], Report No. CB 95/45, 26 September 1997 (unpublished)

1.2 Data protection Yes

1.2.1 Data owner Syngenta

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study This was an investigative study with no applicable guidelines.

2.2 GLP No

2.3 Deviations Not applicable

3 MATERIALS AND METHODS

3.1 Test material

3.1.1 Non-labelled parent compound Fenoxycarb (CGA 114597 tech.)

3.1.1.1 Lot/Batch number [redacted]

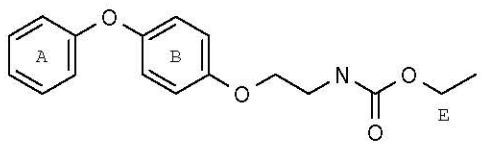
3.1.2 Specification

3.1.2.1 Purity [redacted]

3.1.2.2 Stability In a previous study ([redacted], 1993), the test article was shown to be stable in pelleted diet at concentrations of 10, 100, 1000 and 20000 ppm for at least 5 weeks when stored at room temperature.

Three sub samples of the control and pre-treated food were analysed for content and homogeneity of the test substance. The actual mean test article concentration in the diet was found to be 5323 ppm, which corresponds to 106.5% of the nominal value and thus met the usual quality standards that allow deviations of ±25% from the nominal value. The deviation of individual values of three samples of the food was within ±15% of the mean value and, therefore, the diet batch was considered homogeneous.

3.1.3 Labelled parent compound



Label A: ethyl[2-(4-((ring-U-^{14}C)phenoxy)phenoxy)ethyl]carbamate (labelled in the A-ring)
Batch: JAK-XII-6, chemical purity: [redacted]%, radiochemical purity [redacted]%

Label B: ethyl[2-(4-phenoxy((ring-U-^{14}C)phenoxy))ethyl]carbamate (labelled in the B-ring)
Batch: JAK-XII-4, chemical purity: [redacted]%, radiochemical purity [redacted]%

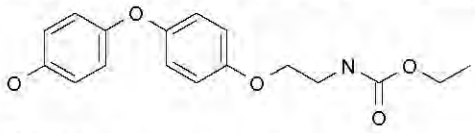
Label E: ethyl-1-^{14}C[2-(4-phenoxyphenoxy)ethyl]carbamate (labelled at the α-carbon of the ethyl group of the ethyl carbamate moiety)

4Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver and lung

Batch: [redacted] chemical purity: [redacted] %, radiochemical purity [redacted] %

Metabolite CGA 294850 used as a substrate was as follows:



^{14}C-CGA 294850, the main metabolite (p-hydroxylation of A-ring) labelled in the A- and B-ring (Label M) – Batch: [redacted], chemical purity: [redacted] %, radiochemical purity [redacted] %

3.2 Test procedure

Lung and liver microsomes from male and female Tif:MAGf(SPF) mice, male and female Tif:RAIf(SPF) rats, male and female marmosets and from humans were incubated with radiolabelled and CGA 294850 (the p-hydroxylated metabolite of fenoxycarb) in the presence of an NADPH-regenerating system to investigate species differences in the *in vitro* metabolism. In addition, lung and liver microsomes from 30 male and 30 female Tif:MAGf(SPF) mice pre-treated for 14 days with 5000 ppm fenoxycarb (admixed to the diet) were investigated. Bodyweight and food consumption of these animals were measured daily during the administration period. After 14 days treatment, the animals were killed and the livers and lungs quickly removed and weighed. The lungs were immediately chopped. The lungs of 10 mice from each group were pooled. The livers of mice were kept individually. All organs were frozen in liquid nitrogen until processed (subcellular fractionation).

For the preparation of the microsomal and cytosolic fractions from rats and marmosets, livers and lungs were collected from 6 male and female Tif:RAIf(SPF) rats each, and from 2 female and 1 male marmoset (Callithrix jacchus), originating from [redacted]. The marmosets were 6, 7 and 9 years old.

Human liver samples (liver nos. KDL 18, 23 and 24 from kidney donors) were provided by [redacted]

Human lung microsomes (Donor nos. 827951L and 1016951L) were obtained from [redacted]).

Microsomal and cytosolic liver fractions from 6 out of 30 mice per group were prepared individually. Microsomal and cytosolic liver fractions from 6 male and female control rats, 1 male and 2 female marmosets, and from 3 humans were prepared. Additionally, microsomes from pooled liver samples were prepared from 6 mice per experimental group, from the 6 male and female rats and from the 3 human livers.

The lungs from ten mice per group were pooled for the preparation of microsomes and cytosol as described above for the liver. Microsomes and cytosol of 6 male and female rat lungs, and of 1 male and 2 female marmoset lungs were prepared. A sample of human lung microsomes was obtained from IIAM.

Biochemical characterisation of the liver and lung subcellular fractions was carried out. Protein contents of liver and lung microsomal and

cytosolic fractions were determined according to Smith et al. [1985].[1] Microsomal 7-ethoxycoumarin O-deethylase activity in liver and lung was determined according to Ullrich and Weber [1972][2] as a marker reaction for cytochrome P450 in order to validate the suitability of the microsomal preparations.

The oxidative metabolism of fenoxycarb and its main metabolite CGA 294850 was investigated in liver as well as lung microsomal fractions from the indicated species in the presence of an NADPH-regenerating system. ^{14}C-fenoxycarb (labels A, B and E) and ^{14}C-CGA 294850 (label M) were used as substrates.

Microsomes were incubated with ^{14}C-radiolabelled fenoxycarb (three labels A, B or C) or ^{14}C-CGA 294850 at a concentration of 100 µmol/L in the presence of NADPH-regenerating system. CGA 294850 was uniformly labelled in both aromatic rings. The incubations were performed at 37°C in a water bath for incubation times between 5 and 45 minutes. The reaction was stopped and the samples deproteinised by addition of 300 µL of a solution of 91% methanol in water containing 3% trichloroacetic acid and precipitated by centrifugation (2000*g*, 10 minutes). The recovery of the radioactivity in the supernatant was determined by liquid scintillation counting. Metabolite analysis was performed with two HPLC systems.

Protein binding of radioactivity was determined after incubation of liver microsomes with ^{14}C-fenoxycarb (label A, B or E) or ^{14}C-CGA 294850 at a concentration of 100 µmol/L as described above. After addition of a stop solution, the samples were centrifuged at 3000 rpm for 15 minutes. The radioactivity in the supernatant was determined by liquid scintillation counting. The pellet was washed twice with methanol (1 mL). The resulting pellet was resuspended and dissolved in 250 µL 1% SDS at about 50°C and the protein subsequently precipitated by the addition of 5 mL of acetone and the step was repeated. The final protein pellet was digested in 1 mL of 2 mol/L NaOH at 50°C, neutralised with 2 mol/L HCl and the radioactivity determined by liquid scintillation counting.

4 RESULTS AND DISCUSSION

4.1 General observations

There were no deaths in this study. No in-life clinical observations were recorded for the animals treated with fenoxycarb. There was no effect of treatment with fenoxycarb on body weight development or food consumption.

4.2 Substance intake

Dietary concentration of fenoxycarb (ppm): 5000

Dose: 1099 / 947 mg/kg bw/day (♂/♀)

4.3 Gross pathology, organ weights, histopathology

There were no treatment-related macroscopic changes observed at necropsy. Treatment was without effect on the absolute and relative lung weights of male and female mice. The absolute and the relative liver weights were increased in treated animals of both sexes to about 140-150% of control (see Table A6_10-1).

[1] Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC. Measurement of protein using bicinchoninic acid. Anal Biochem 1985; 150: 76-85.

[2] Ullrich V and Weber P. The O-dealkylation of 7-ethoxycoumarin by liver microsomes. Hoppe-Seyler's Z Physiol Chem 1972; 353: 1171-1177.

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Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver and lung

4.4	***In vitro* metabolism of ^{14}C-fenoxycarb by lung microsomes**	Lung microsomes from the investigated species had essentially no activity to metabolise fenoxycarb or its main metabolite CGA 294850, even after pre-treatment of mice. Thus, the lung does not seem to play a substantial role for the oxidative metabolism of parent fenoxycarb.
4.5	***In vitro* metabolism of ^{14}C-fenoxycarb by liver microsomes**	Oxidative metabolism of fenoxycarb by liver microsomes was extensive in all four species examined, with over 15 metabolites formed. This *in vitro* metabolism was characterised by remarkable species differences. With respect to disappearance of the parent, the following ranking was established: pre-treated mouse (male or female) > control mouse (male or female) > marmoset ≈ male rat > female rat > man. Pre-treatment of mice with fenoxycarb resulted in about a 2-fold induction of its initial metabolism rate by liver microsomes of both sexes, i.e. in this species the oxidative metabolism of fenoxycarb is subject to auto induction. A somewhat weaker induction of about 1.5-fold was observed with these mouse liver microsomes for the O-deethylation of the cytochrome P450 model substrate 7-ethoxycoumarin. With fenoxycarb, the predominant reaction in all investigated species was the p-hydroxylation to CGA 294850. The initial rate of this reaction was also induced about 2-fold by pre-treatment of mice with fenoxycarb. Further metabolite peaks, obtained with the first HPLC system, resulted from cleavage of one or both rings from fenoxycarb and were identified by LC/MS as the phenolic metabolite arising after removal of the A-ring (CGA 294847), 4,4'-oxybis-phenol (CGA 195935) and 4-phenoxy-phenol (CGA 26021). Of particular interest was CGA 294847, which was formed from fenoxycarb. This metabolite lacks the distal aromatic ring (A-ring), which could have been cleaved off as benzoquinone. Nevertheless, benzoquinone could not be detected as metabolite in incubations with label A. In additional investigations, however, benzoquinone was found to be efficiently transformed to hydroquinone in our incubation system, and indeed, upon incubation of liver microsomes with label A, radioactivity was detected at the chromatographic position of hydroquinone. The formation rates of CGA 294847 and hydroquinone were strongly dependent on the species and on pre-treatment, whereby the following ranking was observed: pre-treated mouse > control mouse > rat ≈ marmoset >> man. Upon incubations with the main metabolite CGA 294850, the distal aromatic ring (A-ring) was cleaved off resulting in the formation of CGA 294847. This further argues for a pathway of O-dearylation of fenoxycarb via p-hydroxylation followed by oxidative cleavage under formation of benzoquinone. A second HPLC system was used to achieve a better separation of the polar metabolites. The chromatograms were particularly analysed for an eventual metabolic formation of ethyl carbamate. Indeed, radioactivity at the retention time of ethyl carbamate could be detected in incubations with label E. The estimated rate of ethyl carbamate formation was also clearly species dependent and found increased in mice upon pre-treatment. The observed ranking was pre-treated male mouse > marmoset > control male mouse ≈ pre-treated female mouse > control female mouse ≈ male or female rat > man.

4Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver and lung

4.6	**Formation of benzoquinone and ethylcarbamate**	1,4-Benzoquinone is an alkylating agent that covalently modifies DNA and protein [Pongracz and Bodell 1990[3]; Thomas et al. 1990][4]. Ethyl carbamate is known to induce lung tumours in mice, rats and hamsters [IARC 1974][5]. Hydroquinone, which was formed from benzoquinone under our in vitro test conditions, and ethyl carbamate were both found in the incubations of fenoxycarb with liver microsomes but not with lung microsomes. However, after formation in the liver, they could be released into the circulation, and due to the relative stability, they could survive transport via the heart to the lung, the susceptible organ. There they might covalently bind to macromolecules, either directly or upon further metabolic activation, and possibly cause tumour formation in this organ upon long term treatment. Among the investigated species the formation of ethyl carbamate and hydroquinone was highest with mouse liver microsomes and even further increased when microsomes from animals pre-treated with fenoxycarb were used. A similar auto induction of the oxidative metabolism can also be assumed for the high doses applied in the mouse long-term studies with fenoxycarb. The formation rates of ethyl carbamate from fenoxycarb, i.e. male mice > female mice > male or female rat, correlate well with the carcinogenic response of fenoxycarb in these two species where lung tumours were observed in male mice at 500 and 2000 ppm, in female mice only at 2000 ppm, but neither in male nor in female rats ([redacted], 1995). When compared with mice and rats, human liver microsomes showed an at least ten-fold lower formation rate of ethyl carbamate and benzoquinone/hydroquinone. This implies that man is more than an order of magnitude less sensitive to lung tumour formation by fenoxycarb via metabolic activation to ethyl carbamate and/or benzoquinone/hydroquinone
4.7	***In vitro* protein binding**	Protein binding with fenoxycarb was observed with all 3 positions of the ^{14}C-label. Binding was higher with A- and B-label than with E-label. Pre-treatment of mice increased microsomal protein binding about 2-fold with label A and B, and about 10-fold with label E. This suggests that in mice pre-treatment specifically induces a metabolic path leading to reactive metabolites containing the E-label.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Results and discussion**	Lung microsomes from mouse, rat, marmoset and man did not metabolise fenoxycarb. With liver microsomes from the same species, fenoxycarb was efficiently metabolised and remarkable, mainly quantitative species differences were observed. The mouse, as the sensitive species for tumour formation with fenoxycarb, exhibited the highest metabolic rate as well as the highest formation rate of metabolites, which might be related to tumour formation, such as ethyl carbamate or hydroquinone/benzoquinone. Man was more than an order of magnitude less efficient in the formation of these metabolites than

[3] Pongracz K and Bodell WJ. Detection of (3'-hydroxy)-1,N^6-benzetheno-2'-deoxyadenosine-3'-phosphate by ^{32}P postlabeling of DNA reacted with p-benzoquinone. Chem Res Toxicol 1990; 4: 199-202.

[4] Thomas DJ, Sadler A, Subrahmanyam VV, Siegel D, Reasor MJ, Wierda D and Ross D. Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid cells. Mol Pharmacol. 1990; 37: 255-262.

[5] IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. IARC Monograph 1974; 7: 111-140.

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Annex Point IIIA VI.7	6.10 In-vitro metabolism by liver and lung

mouse where the lung tumours were observed.

5.2 Conclusion

5.2.1	Reliability	0 - Supplementary data
5.2.2	Deficiencies	None

4Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver and lung

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/09
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1: Pre-treatment of mice: Absolute (g) and relative (%) liver weights

Group	Absolute liver weight	Relative liver weight
Male		
0	1.756	5.784
5000	2.622***	8.048***
Female		
0	1.360	5.467
5000	2.096***	8.132***

*** Statistically significant difference from control group mean, $p<0.001$ (Dunnett's test, 2-sided)

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver microsomes

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]1998), CGA 114597 tech. (Fenoxycarb) - *In Vitro* Formation Of Urethane From Fenoxycarb By Liver Microsomes Of Mouse And Man. [redacted] [redacted]Report No. CB 97/16, 11 June 1998 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Syngenta	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	This was an investigative study with no applicable guidelines.	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Fenoxycarb (CGA 114597 tech.)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Purity	[redacted]	
3.1.2.2	Stability		
3.2	**Test procedure**	Liver microsomal fractions of control mice (Tif:MAGf) and of mice pre-treated with 2000 or 5000 ppm fenoxycarb in the diet, as well as microsomal fractions from three human kidney donor livers (KDL 18, 23 and 24) were used. These microsomal fractions originated from the preceding *in vitro* metabolism study ([redacted], ***1997***), and were shown to be metabolically active using a model substrate as well as with fenoxycarb. Microsomes were incubated with fenoxycarb (^{14}C-radiolabelled for some experiments) at a concentration of 100 µmol/L in the presence of an NADPH-regenerating system. The incubations consisted of liver microsomes (normally 3 mg/mL protein), an NADPH-regenerating system containing final concentrations of glucose-6-phosphate-dehydrogenase (5 µg/mL), NADP (1.31 mmol/L), glucose-6-phosphate (8.04 mmol/L) and $MgCl_2$ (100 mmol/L) and fenoxycarb (100 µmol/L) in a total volume of 800 µL. Incubations were performed at 37°C and the reaction was stopped with 5% perchloric acid. Urethane formation was determined by GC-MS. Propyl carbamate was added as internal standard to the acidified reaction mixture and the protein was precipitated by centrifugation. The supernatant was applied onto a CHEM ELUT solid phase extraction cartridge. The cartridge was washed twice with 4 mL of petroleum ether and urethane and propyl carbamate were eluted with 2 times 4 mL of dichloromethane. To the combined eluate, 500 µL of toluene were added and subsequently evaporated to a volume of 150 to 500 µL in a evaporating centrifuge. The analysis was performed by GC-MS, after electron impact ionisation	X

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Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver microsomes

(EI) as well as after positive chemical ionisation (CI) using NH_3 as reactant.

4 RESULTS AND DISCUSSION

4.1 General observations

In vitro formation of urethane from fenoxycarb in mouse liver microsomal incubations was shown to be dependent on the presence of microsomes and NADPH. Incubation of ethyl-^{14}C-labelled fenoxycarb revealed urethane with a mass of +2 compared with the unlabelled urethane. Thus *in vitro* metabolic release of urethane from fenoxycarb by an oxidative reaction (presumably catalysed by cytochrome P450) was confirmed by GC-MS. Retention time as well as the mass spectra with two ionisation modes electron impact (EI) and chemical ionisation with NH_3 (CI) of *in vitro* metabolically formed urethane were confirmed by comparison with reference material. X

No notable decrease of urethane was observed when the product urethane itself was incubated with mouse liver microsomes in the presence of an NADPH-regenerating system, indicating that with microsomal fractions under the experimental conditions used, urethane is not further metabolised to a substantial extent.

Urethane formation from fenoxycarb by male mouse liver microsomes was time proportional over about 5 minutes, but levelled off afterwards, whereas with human liver microsomes minor amounts of urethane could be observed after 30 minutes, but not after 5 or 10 minutes (see Table A6_10-1).

Protein-dependence was investigated with mouse liver microsomes. Urethane formation was essentially linear with protein up to 2 mg/mL and levelled off at 3 mg/mL.

Urethane formation by liver microsomes of control mice and mice pre-treated with fenoxycarb as well as by microsomes of three human kidney donor livers was compared. Pre-treatment of mice with fenoxycarb resulted in an auto induction of urethane formation by a factor of about 2. Urethane formation was very much lower with the human liver microsomes; even after incubation for 20 or 40 minutes urethane concentrations were below that obtained after one minute with pre-treated mouse liver microsomes. With human liver microsomes, large interindividual differences were observed (see Table A6_10-2).

The specific activities with human liver microsomes were 11- to 173-fold and 22- to 353-fold lower than those with control mice and pre-treated mice liver microsomes, respectively (see Table A6_10-3).

4.2 *In vitro* protein binding

Protein binding with fenoxycarb was observed with all 3 positions of the ^{14}C-label. Binding was higher with A- and B-label than with E-label. Pre-treatment of mice increased microsomal protein binding about 2-fold with label A and B, and about 10-fold with label E. This suggests that in mice pre-treatment specifically induces a metabolic path leading to reactive metabolites containing the E-label. X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Results and discussion

In vitro formation of urethane from fenoxycarb by mouse liver microsomes as observed in a previous study using HPLC analysis was confirmed by GC MS. Urethane formation was induced by pre-treatment of mice with fenoxycarb (auto induction). Remarkable species differences in urethane formation rate between mouse and man were observed. Human liver microsomes were 11- to 173-fold and 22- to 353-fold slower than control and pre-treated male mouse liver

Section A6.10	Mechanistic Studies
Annex Point IIIA VI.7	6.10 In-vitro metabolism by liver microsomes

microsomes, respectively.

5.2 Conclusion

5.2.1	Reliability	0
5.2.2	Deficiencies	None

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 In-vitro metabolism by liver microsomes

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/09
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1: Urethane formation - Results of two parallel incubations for mouse and one incubation for man

Incubation Time [min]	Urethane Concentration in Assay [µmol/L]	
	Mouse	Man
0	0.0 / 0.0	0
2	2.6 / 4.6	nd
5	7.0 / 7.5	0
10	8.0 / 8.1	0
20	5.5 / #	nd
30	8.9 / 6.2	0.43

nd: not determined, # missing value

Table A6_10-2: Urethane formation by microsomal liver fractions of control and pre-treated mice and of 3 human subjects

Incubation Time	Urethane Concentration in Assay [µmol/L]				
	Mouse		Human		
[min]	Control	Pre-treated	Subject 401	Subject 402	Subject 403
0	0	0	0	0	0
1	0.38	1.02			
2	0.98	1.99			
5	2.41	4.15			
20			0.89	0.49	0.06
40			0.57	0.43	0.32

Table A6_10-3: Specific activity of microsomal urethane formation of mouse and human

Species/Sample	Specific activity [pmol/min/mg protein]
Mouse male control	162.7
Mouse male pre-treated (5000 ppm)	331.9
Human Liver 401	14.84
Human Liver 402	8.09
Human Liver 403	0.94
Human Average Liver 401 to 403	7.96

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of DNA-adduct formation in mice

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1 REFERENCE

1.1	**Reference**	[redacted] (1998), CGA 114597 tech. (Fenoxycarb) - Investigation Of The Formation Of Urethane-Derived DNA Adducts In Male Mice. [redacted] Report No. CB 96/48, 16 March 1998 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Syngenta
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	This was an investigative study with no applicable guidelines.
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Non-labelled parent compound	Fenoxycarb (CGA 114597 tech.)
3.1.2	Lot/Batch number	[redacted]
3.1.3	Specification	
3.1.3.1	Purity	[redacted]
3.1.3.2	Stability	Analysis of diet and dosing suspensions for achieved concentration and homogeneity was satisfactory. The test article was found to be homogeneously distributed in the diet at a mean concentration of 2127 ± 37 ppm (106% of the nominal concentration, n=3).
3.1.4	Labelled parent compound	[Ethyl-1-^{14}C]-fenoxycarb
3.1.5	Lot/Batch number	ILS-152.1C (purified batch [redacted])
3.1.6	Specification	
3.1.6.1	Purity	Radiochemical purity [redacted]
3.1.6.2	Spec. activity	0.172 MBq/mg (51.8 GBq/mol)
3.1.7	Reference compound	1-^{14}C-urethane (1-^{14}C-ethyl carbamate); batch CFQ9823; radiochemical purity 98.9%; spec. radioactivity 3.65 MBq/mg (325 GBq/mol)
3.2	**Test procedure**	Two groups of eight young adult male Tif:MAGf(SPF), hybrids of NIHxMAG mice, were treated with either a single oral dose of 440 mg/kg body weight [^{14}C]-fenoxycarb labelled at the ethylcarbamate side chain or 20 mg/kg body weight [^{14}C]-urethane. In order to mimic the pharmacokinetic situation of a long term bioassay, i.e. under conditions where an auto induction of the metabolism of fenoxycarb has been demonstrated, a third group of eight mice was pretreated for 14 days with unlabelled fenoxycarb at a concentration of 2000 ppm in the diet, followed by a single oral dose of 440 mg/kg body weight [^{14}C]-fenoxycarb 2 days after cessation of pretreatment. All animals were sacrificed 24 hours after the administration of the radiolabel. An

additional group of eight mice received a control diet for 17 days and was then sacrificed (control animals).

In order to investigate the dose and time dependence of DNA binding of [^{14}C]-urethane, groups of four mice were treated by a single oral administration of [^{14}C]-urethane at doses of 0, 2, 20 and 40 mg/kg bw and sacrificed after 6 or 24 hours.

Exhaled $^{14}CO_2$ and urinary excretion of radioactivity were measured from four mice.

Individual body weights and food consumption were recorded at regular intervals. At the end of the study the mice were killed, livers and lungs were excised, weighed, washed, minced and frozen at -80°C.

Subcellular liver fractions were prepared and the following biochemical parameters were determined: microsomal protein content, microsomal cytochrome P450 content, lauric acid 11- and 12-hydroxylation.

DNA and chromatin protein were isolated from liver and lung samples and the DNA constituents were analysed. Selected DNA samples were analysed for N7-(2-oxoethyl)-guanine and 1,N^6-etheneoadenine. The covalent binding index was calculated.

4 RESULTS AND DISCUSSION

4.1 General observations

Bodyweight development and food consumption were similar in control animals and in animals treated for 14 days with diet containing 2000 ppm fenoxycarb. The mean daily dose of fenoxycarb was calculated to be 385 mg/kg bw/day.

4.2 Organ weights

Pretreated animals had moderately increased absolute (123% of control) and relative liver weights (121% of control). Animals of group 2 which received control diet during the whole experimental period and were treated with a single oral administration of [^{14}C]-fenoxycarb at a dose of 440 mg/kg body weight showed slightly increased absolute (108% of control) as well as relative liver weights (108% of control), 24 hours after the administration. In animals that received only a single dose of [^{14}C]-urethane (20 mg/kg bw), absolute and relative liver weights were similar to those of untreated mice.

Pretreated animals and animals of group 2, which received control diet during the whole experimental period and were treated with a single oral administration of [^{14}C]-fenoxycarb at a dose of 440 mg/kg body weight, showed similar absolute as well as relative lung weights as control animals. Animals which received only a single dose of [^{14}C]-urethane (20 mg/kg bw) showed slightly increased absolute (115% of control) as well as relative lung weights (114% of control), 24 hours after the administration (see Table A6_10-1).

4.3 Biochemical parameters

Pretreatment for 14 days with unlabelled fenoxycarb followed by a single oral dose of 440 mg/kg body weight [^{14}C]-fenoxycarb showed the expected peroxisome proliferator type of liver enzyme induction (increased microsomal protein content, doubling of the microsomal cytochrome P450 content and increases of 480% and 860% of lauric acid 11- and 12-hydroxylation, respectively). A qualitatively similar but weaker enzyme induction was observed after treatment with a single dose of [^{14}C]-fenoxycarb. Treatment with a single dose of [^{14}C]-urethane was without an effect on the investigated biochemical parameters (see Table A6_10-2).

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of DNA-adduct formation in mice

4.4	**DNA adduct formation of urethane**	Exhaled $^{14}CO_2$, collected during 24 hours after treatment with 20 mg/kg body weight [^{14}C]-urethane contained 28.7%, and the urine sample collected for 24 hours after treatment contained 7.11% of the total radioactivity administered (see Table A6_10-3). Radioactivity was clearly detectable in all DNA samples isolated from the livers, 24 hours after single oral administration of 20 mg/kg bw [^{14}C]-urethane. A slightly higher specific radioactivity was found in the DNA sample isolated from lungs (see Table A6_10-4). Upon HPLC analysis of the desoxyribonucleotides from the livers, 62% of the total DNA radioactivity eluted in the fractions containing the normal nucleotides and was attributed to biosynthetic incorporation, whereas 38% eluted in the region known to contain putative DNA-test compound adducts. The value of 38% corresponded to a CBI (Covalent Binding Index, CBI = µmol chemical bound per mol DNA nucleotide / mmol chemical applied per kg body weight) of 0.8. Upon HPLC analysis of the DNA purines from liver DNA samples, 17-27% and 2.1-3.8% of the total DNA radioactivity eluted in the fractions containing 7OEG and εA, respectively. Expressed in the units of the CBI, these radioactivities corresponded to 7OEG- and εA-adduct levels of 0.5-0.8 and 0.07-0.1, respectively. Due to the low amount of DNA available from the lung no HPLC analysis of the desoxyribonucleotides was performed. Upon HPLC analysis of the DNA purines from lung DNA, about 22% of the total DNA radioactivity could be attributed to 7OEG whereas no radioactivity was detectable in the fractions containing εA. The respective 7OEG- and εA-adduct levels in lung DNA, expressed in the units of the CBI, were 0.7 and less than 0.1.
4.5	**Time and dose dependence of liver DNA adduct formation of urethane**	With [^{14}C]-urethane a largely dose-proportional increase of the specific DNA radioactivity in liver was observed between 2 and 20 mg/kg bw, 24 hours after the administration, whereas at 40 mg/kg bw a saturation of the specific DNA radioactivity was discernible. No HPLC analysis of the desoxyribonucleotides was performed. 7OEG- and εA-adduct levels in liver DNA were essentially proportional to the dose at 20 and 40 mg/kg bw. Due to the low specific DNA radioactivity after treatment with 2 mg/kg bw no HPLC analysis of the purines could be performed. A clearly higher specific DNA radioactivity was found 24 hours after the administration of 20 mg/kg bw [^{14}C]-urethane than 6 hours after (see Table A6_10-5).
4.6	**DNA adduct formation of fenoxycarb**	Exhaled $^{14}CO_2$ and urine, collected in the first 24 hours after treatment with [^{14}C]-CGA 114597, contained 2%-3% and 44-49% of the administered radioactivity, respectively. Pre-treatment with 2000 ppm unlabelled fenoxycarb was without an effect on exhalation of [^{14}C]O_2 and urinary excretion of the radiolabel. In all DNA samples isolated from [^{14}C]-fenoxycarb treated animals radioactivity was clearly detectable. In DNA samples isolated from the livers of animals pre-treated with unlabelled fenoxycarb, substantially lower specific DNA radioactivities were found compared with the respective DNA samples from the animals that were not pre-treated. Specific radioactivities were similar in DNA samples from the lungs of pretreated and non pre-treated animals, and were in the range of those in the livers of pre-treated mice.

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of DNA-adduct formation in mice

HPLC analysis of the desoxyribonucleotides from the livers of [^{14}C]-fenoxycarb treated mice revealed that the DNA radioactivity was completely due to biosynthetic incorporation. No radioactivity eluted in the region known to contain the more lipophilic DNA-test compound adducts at a limit of detection of 0.1 in the units of the CBI.

In addition, no urethane-type DNA adducts 7OEG and εA were detectable upon analysis of the liver DNA purines at a limit of detection of 0.09 in the units of the CBI.

Due to the low specific activity and due to the low amount of DNA available, no analysis of the desoxynucleotides and of the content of 7OEG and εA could be performed with lung DNA.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Results and discussion

The results of the present study give no indication for a genotoxic potential of fenoxycarb mediated by the formation of urethane-type DNA adducts in the mouse. A maximum possible release of urethane from fenoxycarb of less than 2.8% can be calculated based on the limit of detection for 7OEG in liver DNA after treatment with [^{14}C]-fenoxycarb and the 7OEG-levels obtained in liver DNA after treatment with [^{14}C]-urethane.

The maximum possible CBI for fenoxycarb is only 0.1 or at least 10 times lower as that typically found with weak genotoxic chemicals. From a quantitative analysis of DNA binding and tumourigenicity data for well known genotoxic carcinogens, one would expect a CBI of around 13 to explain the tumour formation in mice by fenoxycarb as observed in the long-term bioassay, if DNA binding was the mechanism of tumourigenic action. However, the observed DNA-binding potency of fenoxycarb is more than two orders of magnitude lower.

Therefore it is highly unlikely, that genotoxicity mediated by DNA binding of metabolically released urethane is the mechanism for tumourigenicity of fenoxycarb.

5.2 Conclusion

5.2.1 Reliability 0 - Supplementary data

5.2.2 Deficiencies None

Section A6.10 Mechanistic Studies

Annex Point IIIA VI.7 6.10 Investigation of DNA-adduct formation in mice

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/09
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1: Intergroup comparison of organ weights

Pre-treatment	Fenoxycarb 2000 ppm, 14 d	No pre-treatment	No pre-treatment	No pre-treatment
Treatment	[^{14}C]-Fenoxycarb	[^{14}C]-Fenoxycarb	[^{14}C]-Urethane	none
Liver weight (g)	2.45	2.14	1.94	1.98
Relative liver weight (% bw)	6.88	6.13	5.54	5.69
Lung weight (g)	0.215	0.207	0.234	0.204
Relative lung weight (% bw)	0.606	0.596	0.666	0.585

Table A6_10-2: Intergroup comparison of mean biochemical parameters

Pre-treatment	Fenoxycarb 2000 ppm, 14d	No pre-treatment	No pre-treatment	No pre-treatment
Treatment	[^{14}C]-Fenoxycarb	[^{14}C]-Fenoxycarb	[^{14}C]-Urethane	none
Microsomal protein (mg/g liver)	28.68	21.59	17.69	18.29
Cytochrome P450 (nmol/g liver)	31.00	23.87	12.77	14.4
Lauric acid 11-hydroxylase (nmol/min/g liver)	50.91	29.99	12.23	10.71
Lauric acid 12-hydroxylase (nmol/min/g liver)	130.6	98.4	15.2	15.1

Table A6_10-3: Urinary excretion and exhalation of radioactivity within 24 hours of single dose of [^{14}C]-fenoxycarb and [^{14}C]-urethane

Pre-treatment	Fenoxycarb 2000 ppm, 14d	No pre-treatment	No pre-treatment
Treatment	[^{14}C]-Fenoxycarb	[^{14}C]-Fenoxycarb	[^{14}C]-Urethane
% of administered radioactivity excreted:			
In urine	44.1	48.4	7.11
Exhaled as [^{14}C]O_2	2.12	2.71	28.7

Table A6_10-4: Radioactivity in liver and lung DNA isolated 24 hours after administration of [^{14}C]-urethane to mice (CBI units = µmol chemical bound per mol DNA nucleotide / mmol chemical applied per kg body weight)

	[^{14}C]urethane	[^{14}C]urethane	Control	Control
Nominal dose (mg/kg bw)	**20**	**20**	**0**	**0**
LIVER:				
Chromatin protein (dpm/mg)	918	1265	-	131
DNA amount in vial (mg)	0.619	0.767	1.98	0.598
Gross activity (cpm)	23.6	29.9	11.2	11.5
Specific activity (dpm/mg)	25.7	31.3	-	-
Binding to DNA	1.74	2.09	-	-
After repurification	1.76	n.d.	-	-
Obtained from nucleotide analysis	n.d.	0.8	-	-
7OEG-Level	0.5	n.d	-	-
εA-Level	0.07	n.d	-	-
LUNG:				
Chromatin protein (dpm/mg)	1122		-	
DNA amount in vial (mg)	1.84		1.44	
Gross activity (cpm)	77.6		10.8	
Specific activity (dpm/mg)	46.5		-	
Binding to DNA	3.12		-	
Obtained from nucleotide analysis	n.d.		-	
7OEG-Level	0.7		-	
εA-Level	<0.1		-	

nd: not determined

Table A6_10-5: Dose and time dependence of liver DNA binding of [^{14}C]-urethane - (CBI units)

Nominal dose (mg/kg bw)	**40**	**20**	**2**	**20**	**0**
Sacrifice (hours after administration)	**24**	**24**	**24**	**6**	**24**
Binding to DNA	2.85	4.94	3.61	0.704	-
7OEG-Level	0.89	0.83	n.d.	n.d.	-
εA-Level	0.09	<0.1	n.d.	n.d.	-

n.d. not determined
CBI = µmol chemical bound per mol DNA nucleotide / mmol chemical applied per kg body weight

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

			Official use only
		1 REFERENCE	
1.1	**Reference**	PPP Dossier on Fenoxycarb, Section 5.9.1	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Syngenta	
1.2.2	Company with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 SUMMARY	
3.1	**Occupational Health Surveillance Programs**	**General** Manufacturing employees in Switzerland are medically examined by company physicians at the beginning of their employment and then routinely every 2 years up to the age of 45, then once a year. In Switzerland, routine medical examinations according to the criteria of the Swiss Accident Insurance Institution (SUVA) include: – Anamnesis, – Physical examination: blood pressure, X-ray, hearing test, vision tests, clinical medical examination – Blood analysis: haemoglobin, erythrocytes, leukocytes, thrombocytes, complete blood count, blood sedimentation rate, blood sugar, cholesterol, triglycerides, ALAT, ASAT, alkaline phosphatase, bilirubin, creatinine, uric acid – Urine analysis If needed, additional specific biological investigations included urine Hg (twice a year), and cholinesterase (twice a year). Employees in other countries also are medically examined according to nationally established guidelines.	
3.2	**Data from persons exposed in manufacturing**	Questionnaires for health records have been sent to the heads of the production sites and the responsible occupational physicians: Fenoxycarb (CGA 114597) is an insecticide that acts by contact and ingestion. It disrupts the insect transformations and therefore has the same biological effect as natural insect growth regulators. Fenoxycarb is manufactured by [redacted] and is formulated [redacted] Data was sought in September 2003 from all sites handling either the technical active ingredient or formulated product. [redacted] Fenoxycarb has been handled in [redacted] from 1989 - 1990 and 1996 - 2003. There are many temporary people working in this building (up to 40) with frequent changes. It is not possible to give an exact number of people who have been in contact with fenoxycarb but the number exceeds 200. No adverse effects associated with fenoxycarb handling have been	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

observed to date.

[redacted]

Fenoxycarb has been handled at this site since 1995, [redacted] producing [redacted] of formulated product per run. No adverse reactions associated with fenoxycarb have been reported.

4 APPLICANT'S SUMMARY AND CONCLUSION

In conclusion, large quantities of fenoxycarb, (as formulated products), have been handled within Syngenta (and its legacy companies) for over 15 years, without any associated adverse health effects of the workforce being noted. This is in concordance with the benign toxicity profile of the material.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/30
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.2 6.12.2 Direct observations, e.g. clinical cases and poisoning incidents

			Official use only
		1 REFERENCE	
1.1	**Reference**	PPP Dossier on Fenoxycarb, Section 5.9.2, p. 29	X
1.2	**Data protection**	No	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 APPLICANT'S SUMMARY AND CONCLUSION	
		No cases of poisoning have been reported to the company. No reports of poisoning cases from the open medical literature are on record.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/30
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.3 6.12.3 Health records, both from industry and other available sources

			Official use only
		1 REFERENCE	
1.1	**Reference**	PPP Dossier on Fenoxycarb, Section 5.9.1	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Syngenta	
1.2.2	Company with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 SUMMARY	
3.1	**Occupational Health Surveillance Programs**	**General** Manufacturing employees in [redacted] are medically examined by company physicians at the beginning of their employment and then routinely every 2 years up to the age of 45, then once a year. In [redacted], routine medical examinations according to the criteria of the [redacted] include: – Anamnesis, – Physical examination: blood pressure, X-ray, hearing test, vision tests, clinical medical examination – Blood analysis: haemoglobin, erythrocytes, leukocytes, thrombocytes, complete blood count, blood sedimentation rate, blood sugar, cholesterol, triglycerides, ALAT, ASAT, alkaline phosphatase, bilirubin, creatinine, uric acid – Urine analysis If needed, additional specific biological investigations included urine Hg (twice a year), and cholinesterase (twice a year). Employees in other countries also are medically examined according to nationally established guidelines.	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.3 6.12.3 Health records, both from industry and other available sources

3.2 Data from persons exposed in manufacturing

Questionnaires for health records have been sent to the heads of the production sites and the responsible occupational physicians:

Fenoxycarb (CGA 114597) is an insecticide that acts by contact and ingestion. It disrupts the insect transformations and therefore has the same biological effect as natural insect growth regulators.

Fenoxycarb is manufactured by [redacted] and is formulated at [redacted]

Data was sought in September 2003 from all sites handling either the technical active ingredient or formulated product.

[redacted]

Fenoxycarb has been handled in [redacted] from 1989 - 1990 and 1996 - 2003. There are many temporary people working in this building ([redacted]) with frequent changes. It is not possible to give an exact number of people who have been in contact with fenoxycarb but the number exceeds [redacted]

No adverse effects associated with fenoxycarb handling have been observed to date.

[redacted]

Fenoxycarb has been handled at this site since 1995, typically [redacted] production runs per year, producing [redacted] of formulated product per run. No adverse reactions associated with fenoxycarb have been reported.

4 APPLICANT'S SUMMARY AND CONCLUSION

In conclusion, large quantities of fenoxycarb, (as formulated products), have been handled within Syngenta (and its legacy companies) for over 15 years, without any associated adverse health effects of the workforce being noted. This is in concordance with the benign toxicity profile of the material.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/30
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.3 6.12.3 Health records, both from industry and other available sources

Remarks

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.4 6.12.4 Observations on exposure of the general population and epidemiological studies

Official use only

1 REFERENCE

1.1 Reference PPP Dossier on Fenoxycarb, Section 5.9.3, p. 29

1.2 Data protection No

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 APPLICANT'S SUMMARY AND CONCLUSION

No epidemiological study has been performed by the company. To our best knowledge, there is no other available source of epidemiological data on fenoxycarb.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2007/03/30

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

Remarks [redacted]

COMMENTS FROM ... *(specify)*

Date *Give date of comments submitted*

Materials and Methods *Discuss if deviating from view of rapporteur member state*

Results and discussion *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Remarks

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.5 6.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available

			Official use only
		1 REFERENCE	
1.1	**Reference**	PPP Dossier on Fenoxycarb, Section 5.9.4	X
1.2	**Data protection**	No	

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 APPLICANT'S SUMMARY AND CONCLUSION

Specific Signs of Poisoning

Fenoxycarb is of low acute toxicity. Poisonings are only conceivable if extremely large quantities are ingested. Possible signs of poisoning are non-specific.

Clinical Tests

No specific tests in humans have been performed.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/30
Materials and Methods	[redacted]ion
Results and discussion	[redacted].
Conclusion	[redacted]
Remarks	[redacted]e
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.6 6.12.6 Sensitisation/allergenicity observations, if available

Official use only

1 REFERENCE

1.1 Reference Not applicable

1.2 Data protection No

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 APPLICANT'S SUMMARY AND CONCLUSION

A PubMed search was conducted on February 20, 2006 with the following search strings:

[fenoxycarb] AND [sensitisation]

[fenoxycarb] AND [sensitization]

[fenoxycarb] AND [allergy]

[fenoxycarb] AND [hypersensitivity]

No hits were achieved with either string.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/30
Materials and Methods	[redacted].
Results and discussion	[redacted].
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12 Medical data in anonymous form

Annex Point IIA VI.6.9.7 6.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known

Official use only

1 REFERENCE

1.1 Reference PPP Dossier on Fenoxycarb, Section 5.9.4

1.2 Data protection No

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 APPLICANT'S SUMMARY AND CONCLUSION

3.1 First Aid Measures

Terminate exposure, remove person from scene of spillage or other contamination.

In case of skin contact: Remove contaminated clothing and thoroughly wash the affected parts of the body with soap and water.

In case of eye contact: Rinse eyes with clean water for several minutes. Obtain medical advice.

In case of ingestion: Administer medicinal charcoal in large quantity of water. Obtain medical advice.

3.2 Antidote and Medical Treatment

No antidote is known, apply symptomatic treatment.

In case of skin/eye contact:: Decontamination

In case of ingestion: If the amount ingested is judged less than a potentially toxic dose, employ general supportive measures only. Use activated charcoal for gastrointestinal decontamination. If gastric lavage is considered necessary, prevent aspiration.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/08/10
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Britt, T. (1994): Hydrolysis of ^{14}C-Fenoxycarb at pH 5, 7 and 9, McKenzie Laboratories, Inc., Phoenix, United States, unpublished report, Proj. No RC-0001. Study dates: February - August 1993. Issue date: 08 March, 1994. (Syngenta File No. CGA114597/0468)	
1.2	**Data protection**	No	
1.2.1	Data owner	Syngenta Crop Protection AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 161-1: "Hydrolysis Studies", U.S. Environmental Protection Agency, October 18, 1982.	X
2.2	**GLP**	Yes (McKenzie Laboratories, Inc., Phoenix, United States)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Active Substance: ISO common name: fenoxycarb; Company Code: CGA 114597 Chemical name: [2-(4-phenoxy-phenoxy)-ethyl]-carbamic acid ethyl ester Radiolabeled test substance: phenyl ring "B"-U-^{14}C-labelled fenoxycarb	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Specific radioactivity: 32.5 µCi/mg	
3.1.3	Purity	Radiochemical purity: [redacted]	
3.1.4	Further relevant properties	Water solubility of fenoxycarb: 7.9 mg/l at 25 °C (Stulz, 1993), beige wax solid	
3.2	**Reference substance**	Fenoxycarb (unlabeled): Lot no. S91-1536, purity: 99.0%	
3.2.1	Initial concentration of reference substance	-	
3.3	**Test solution**	The study was carried out with buffer solutions at three pH levels: - pH 5: 0.01 M acetic acid – sodium acetate - pH 7: 0.01 M sodium dihydrogen phosphate – disodium hydrogen - pH 9: 0.01 M sodium borate – hydrochloric acid Concentrations range of radiolabeled fenoxycarb: between 2.0 - 2.1 mg a.s./l.	X

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

3.4	**Testing procedure**		
3.4.1	Test system	See Point 3.3	
3.4.2	Temperature	Experiments were run at 25±1 °C.	X
3.4.3	pH	5 / 7 / 9	X
3.4.4	Duration of the test	30 days	
3.4.5	Number of replicates	Two vessels were investigated at each sampling time for each pH level	
3.4.6	Sampling	pH 5 and 7: 0, 1, 3, 7, 10, 15, 20, 24, 30 days pH 9: 0, 1, 3, 7, 10, 17, 20, 24, 30 days	
3.4.7	Analytical methods	Test solutions were investigated by liquid scintillation counting (LSC, material balance), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) LSC: each sample was counted using a single label disintegration per minute (DPM) data calculation program. LSC efficiency was computed for each sample by fitting its spectrum to a library reference spectrum. HPLC: Column: Supelcosil LC-18 reverse phase, 5 µm, 250 x 4.6 mm; Mobile phase: acetonitrile/ HPLC grade water, linear gradient system Flow rate: 1.0 ml/min. Column temperature: 35°C Detector: UV, 235 nm Injection volume: 50 µl TLC: 2D-TLC Plates: precoated with silica gel 60 F-254 at 0.25 mm thickness Solvent systems: I: methylene chloride:methanol 90:10 (v:v) II: chloroform:glacial acid 90:10 (v:v)	
3.5	**Preliminary test**	None	
		4 RESULTS	
4.1	**Concentration and hydrolysis values**	See Table A7_1_1_1_1-1	
4.2	**Hydrolysis rate constant (k_h)**	pH 5: 4.93×10^{-4} day^{-1}, $r^2 = 0.7106$ pH 7: 2.21×10^{-4} day^{-1}, $r^2 = 0.353$ pH 9 -0.169×10^{-4} day^{-1}, $r^2 = 0.118$ The correlation coefficients indicate that fenoxycarb is hydrolytically stable	
4.3	**Dissipation time**	pH 5: 1406 days pH 7: 3136 days pH 9: 4101 days	
4.4	**Concentration – time data**	Concentrations of the test substance expressed as percentage of the total radioactivity (HPLC, normalised) are given in Table A7_1_1_1_1-1	

Section A7.1.1.1.1 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA7.6.2.1

4.5	**Specification of the transformation products**	Only minimal degradation was observed. No major degradate (≥10% of the applied radioactivity) was formed under pH 5, 7 and 9 conditions. One minor degradate was detected in the 13 minute HPLC eluant of several test solutions (max. 6.24% at pH 9, day 10, replicate 1). Its occurrence was postulated as being erratic. The unknown was interpreted as contaminant or impurity that was transient and possibly adheres to glassware.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In this study, the significance of hydrolysis as a route for degradation of fenoxycarb was established. The radiolabelled test substance was incubated in sterilised aqueous buffered solutions of pH 5, 7 and 9 at 25 °C in the dark at concentrations ranging between 2.0 and 2.1 mg/l. Duration: 30 days
5.2	**Results and discussion**	The parent concentrations were essentially stable under pH 5, 7 and 9 conditions for the entire 30 day study period. For all pH's and all sampling intervals tested, fenoxycarb accounted for greater than or equal to 95% of the applied dose at day 0.
5.2.1	k_h	A value for k_h is given in the report however, since only insignificant degradation is observable the reporting is not meaningful
5.2.2	DT_{50}	A value for DT_{50} is given in the report however, since only insignificant degradation is observable the reporting is not meaningful
5.2.3	r^2	Correlation coefficients between 0.118 and 0.7106 are given, indicating that fenoxycarb is hydrolytically stable
5.3	**Conclusion**	It was concluded that hydrolysis would not be expected to be a major degradation pathway for fenoxycarb.
5.3.1	Reliability	1
5.3.2	Deficiencies	None.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/07/03
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_1_1-1: Hydrolysis of fenoxycarb at pH 5, 7 and 9, respectively after different incubation times (HPLC measurement of fenoxycarb in percent of total radioactivity, normalised)

Incubation time (d)	% of total fenoxycarb radioactivity, normalized		
	pH 5	pH 7	pH 9
0	99.10	100.0	97.74
1	99.48	99.02	99.64
3	99.66	99.49	99.16
7	100.00	98.74	97.56
10	99.22	98.70	95.44
15/17	98.67	99.10	99.26
20	99.12	98.80	98.78
24	99.14	97.78	99.10
30	97.44	99.60	98.54

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products (1)

			Official use only
		1 REFERENCE	
1.1	**Reference**	1) Clark, A. (1994): Photodegradation of ^{14}C-Fenoxycarb (Phenyl-^{14}C-CGA-114597) in pH 7 buffered solution under artificial sunlight. Ciba-Geigy Corp., Greensboro, United States, unpublished report No.ABR-94071. Issue date: 19 December, 1994. (Syngenta File No. CGA114597/0513) 2) Clark, A., Phelps, L. and Cruz, S. (1995): Photodegradation of ^{14}C-Fenoxycarb (Phenyl-^{14}C-CGA-114597) in pH 7 buffered solution under artificial sunlight. Ciba-Geigy Corp., Greensboro, United States, unpublished report No.ABR-94072. Issue date: 18 January 1995. (Syngenta File No. CGA114597/0529)	
1.2	**Data protection**	No	
1.2.1	Data owner	Syngenta Crop Protection AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982.	X
2.2	**GLP**	Yes (Ciba Plant Protection, Greensboro, NC 27419).	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Active Substance: ISO common name: fenoxycarb; Company Code: CGA 114597 Chemical name: [2-(4-phenoxy-phenoxy)-ethyl]-carbamic acid ethyl ester Radiolabeled test substance: 1) Phenyl ring "A"-U-^{14}C-labelled fenoxycarb 2) Phenyl ring "B"-U-^{14}C-labelled fenoxycarb	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	1) Specific radioactivity: 4.16 MBq/mg 2) Specific radioactivity: 7.31 MBq/mg	
3.1.3	Purity	[redacted]	

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (1)

Annex Point IIA7.6.2.2

3.1.4	Radiolabelling	Yes; see points 3.1.2 and 3.1.3
3.1.5	UV/VIS absorption spectra and absorbance value	Adsorption of fenoxycarb between 290 and 320 nm
3.1.6	Further relevant properties	Water solubility of fenoxycarb: 7.9 mg/l at 25 °C (Stulz, 1993)
3.2	**Reference substances**	Non-radiolabeled fenoxycarb in 1) and 2): Lot no. S91-1536, purity 99%
3.3	**Test solution**	Aqueous solutions of ^{14}C-labelled fenoxycarb (approximately 1 mg a.s./L) in pH 7 phosphate buffer
3.4	**Testing procedure**	
3.4.1	Test system	Test solutions were irradiated with xenon arc light at an average of 1.14 x 10^{-2} Watts/cm^2 under sterile conditions. Samples were exposed to light for 12 hours per day followed by 12 hours dark intervals. Volatiles were collected and samples assayed for radiochemical balance and degradation pattern. Dark controls were run for the same time intervals under same conditions but protected from light.
3.4.2	Properties of light source	Heraeus Suntest Accelerated Exposure Unit: Xenon arc light source equipped with pre-aged borosilicate UV glass filters allowing no light <290 nm to reach the samples
3.4.3	Determination of irradiance	Intensity: The intensity of the lamp was measured at the start and end of the study. It was set to 410 W/m^2 on the Radialux unit. Spectral distribution: The spectral distribution was measured with an IL-1700, IL 760 and a Kratos drive. The distribution, 200-700 nm, of the xenon arc lamp is compared to the distribution of natural sunlight
3.4.4	Temperature	25 ± 1 °C
3.4.5	pH	pH 7
3.4.6	Duration of the test	Total incubation time / duration of the study: 30 days
3.4.7	Number of replicates	Replicate samples were investigated for each sampling interval and condition
3.4.8	Sampling	1) Samples were taken at intervals of 0, 2, 4, 7, 14, 22 and 30 days for both, irradiated and dark control test runs 2) Samples were taken at intervals of 0, 4, 7, 14, 21 and 30 days for irradiated and 0. 14 and 30 days for non-irradiated test runs
3.4.9	Analytical methods	LSC: all ^{14}C measurements were made using a Beckman LS 3801 or 5000 liquid scintillation counter according to an internal SOP. LSC efficiency was computed for each sample using external standard channel ration values and the quench fitting to previously established quench standard sample data. UV-VIS spectra have been determined by a Spectronic 3000 array spectrometer HPLC Column: YMC-Pack Polymer-C18, reverse phase, 6 µm, 250 x 4.6 mm was attached to Lichrosorb Diol, 5 µm (250 x 4.6 mm); all columns were preceded by a YMC-C18 guard cartridge

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (1)

Annex Point IIA7.6.2.2

		Mobile phase: acidified water/acetonitrile/methanol, gradient system Flow rate: 1.0 ml/min. Detector: UV, 254 nm TLC: 2D-TLC, normal phase Plates: precoated with silica gel 60 F-254 at 0.25 mm thickness, four solvent systems	
3.5	**Transformation products**	Yes; <u>Degradation products > 10% of the initial dose:</u> 1) Phenol: Plateau between 17.9% and 17.4% by days 22 and 30. 2) CGA 294847: Increased to 10.4% on day 14, then reached 13.4% and 16.9% by days 21 and 30. The presence of phenol shows that biphenyl ether bridge cleavage has occurred. <u>Other minor degradation products</u>: 1) CGA 294850 (< 3.6%), CGA 26021 (< 5.8%) and 1,2-dihydroxy benzoquinone. Catechol, resorcinol and CGA 294851 were tentatively characterised. 2) CGA 294850 (6.9%), CGA 26021 (7.1%) and CGA 294851 (1.6%).	
3.5.1	Method of analysis for transformation products	HPLC, TLC (*cf.* Point 3.4.9) and GC-MS in the Electron Impact mode	
		4 RESULTS	
4.1	**Screening test**	Not performed	
4.2	**Actinometer data**	No data	X
4.3	**Controls**	Fenoxycarb under non-irradiated conditions in aqueous solution was stable with no significant degradation detectable. The parent remained between 96.6% and 98.2% in study 1) and 93.1% and 98.3% in study 2) through day 30	
4.4	**Photolysis data**		
4.4.1	Concentration values	1) 1.28 – 1.43 mg/L 2) 1.26 – 1.43 mg/L	
4.4.2	Mass balance	Radiochemical balance 1) 94.7% to 99.8% (irradiated samples); 97.3% to 100% (dark controls) 2) 92.4% to 100% (irradiated samples); 98.7% to 100% (dark controls)	
4.4.3	k_p	1) -0.039 (r^2 = -0.981) 2) -0.030 (r^2 = -0.979)	
4.4.4	Kinetic order	Pseudo-first order kinetics	
4.4.5	k^c_p / k^a_p	n.a.	X
4.4.6	Reaction quantum yield (ϕ^c_E)	Not determined	X

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (1)

Annex Point IIA7.6.2.2

4.4.7	k_{pE}	n.a.	X
4.4.8	Half-life ($t_{1/2E}$)	1) 18.0 days 2) 23.1 days	
4.5	**Specification of the transformation products**	See Point 3.5	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Photodegradation studies in water have been conducted according to the guideline (see point 2.1).	
5.2	**Results and discussion**	Fenoxycarb is susceptible to aqueous photolysis as could be demonstrated by the results of the two studies. The test substance degraded with half-lives between 18 and 23 days in pH 7 buffered aqueous solution. The spectral distribution of the xenon lamp through filters is compared with natural sunlight. It is shown that in the range between 290 and 400 nm the light intensity of the light source used for sample irradiation in the set up of this study has only about half the intensity of natural sunlight. In the range of overlap of the irradiation with the adsorption of fenoxycarb between 290 and 320 nm the intensity of the irradiated light was very low. Samples were exposed to the xenon light in inverted samples vials. This set-up is relatively simple but the amount of incident light is not defined due to undefined optical path and reflections. Therefore the incident light in the sample vials can be assumed to be significantly lower than the measured value. As a consequence, the photolytic degradation of fenoxycarb under the conditions described in the study can be expected to be relatively low, leading to long photolytic half-lives.	X
5.2.1	k_p	1) -0.039 (r^2 = -0.981) 2) -0.030 (r^2 = -0.979)	
5.2.2	K_{pE}	n.a.	X
5.2.3	ϕ^c_E	n.a.	X
5.2.4	$t_{1/2E}$	Fenoxycarb degrades under photolytic conditions in the pH 7 buffered aqueous solution following experimental half-lives of 1) 18 days and 2) 23 days. Under non-irradiated control or hydrolytic conditions at pH 7 fenoxycarb did not degrade significantly.	
5.3	**Conclusion**	The photolytic half-lives determined under the conditions described are not easily comparable with natural conditions in the environment. The light intensity available for photolytic reaction in the experimental setups is significantly lower than natural sunlight. Therefore the half-lives of 18 and 23 days as determined in the studies can be considered as worst-case values.	
5.3.1	Reliability	1	X

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (1)

Annex Point IIA7.6.2.2

5.3.2 Deficiencies No

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/09
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products (2)

			Official use only
		1 REFERENCE	
1.1	**Reference**	Sack, S. (1991): CGA 114597 (Ro-5223/045), Photodegradation studies in aqueous solution, Dr. R. Maag Ltd., Dielsdorf, Switzerland, unpublished report No.RES-MET-J58. Study dates: June - December 1990. Issue date: April 30, 1991. (Syngenta File No. CGA114597/0109)	
1.2	**Data protection**	No	
1.2.1	Data owner	Syngenta Crop Protection AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982.	X
2.2	**GLP**	Yes (Dr. R. Maag Ltd., CH-8157 Dielsdorf, Switzerland)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Active Substance: ISO common name: fenoxycarb; Company Code: CGA 114597 Chemical name: [2-(4-phenoxy-phenoxy)-ethyl]-carbamic acid ethyl ester Radiolabeled test substance: phenyl ring "B"-U-^{14}C-labelled fenoxycarb	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Specific radioactivity: 1.19 MBq/mg	
3.1.3	Purity	[redacted]	
3.1.4	Radiolabelling	Yes; see points 3.1.2 and 3.1.3	
3.1.5	UV/VIS absorption spectra and absorbance value	UV absorption spectra were measured with a double beam spectrometer	
3.1.6	Further relevant properties	Water solubility of fenoxycarb: 7.9 mg/l at 25 °C (Stulz, 1993)	
3.2	**Reference substances**	Unlabeled fenoxycarb, batch no. 22028/Maag February 1988, purity 99.3%	

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (2)

Annex Point IIA7.6.2.2

3.3	**Test solution**	Aqueous solutions of ^{14}C-labelled fenoxycarb (1.0 mg/l) in 0.15 M phosphate buffer at pH 7 (unsensitized) or using acetone as a sensitizer
3.4	**Testing procedure**	
3.4.1	Test system	Test solutions were irradiated with xenon arc light under sterile conditions. The emission spectrum of the artificial light was shown to be comparable to that of natural sunlight, both revealing a cut-off below 300 nm. Volatiles were collected and samples assayed for radiochemical balance and degradation pattern. Dark control, wrapped samples were incubated for 6 hours under the same conditions. A test series with 2% acetone as a sensitizer was run under the same conditions.
3.4.2	Properties of light source	Hanau SUNTEST equipment fitted with a xenon arc lamp. The equipment has a built-in filter to cut off any radiation with a wavelength <290 nm
3.4.3	Determination of irradiance	The absolute incident light intensity was determined using a p-nitroanisole-pyridine actinometer
3.4.4	Temperature	24 to 26 °C
3.4.5	pH	pH 7
3.4.6	Duration of the test	Total incubation time / duration of the study: 6 hours
3.4.7	Number of replicates	Duplicate samples were exposed to light.
3.4.8	Sampling	Irradiated samples: after 0, 2, 3, 4, 5 and 6 hours (unsensitized) after 0, 1, 1.5, 2, 2.5 and 3.5 hours (sensitized) Dark control samples: after 0 and 6 hours (unsensitized) after 0 and 3.5 hours (sensitized)
3.4.9	Analytical methods	Photolysis solutions were worked up by liquid extraction with ethylacetate and analysed by TLC, HPLC and MS. Volatile radioactivity trapped in polyurethane plugs was extracted with methanol and analysed by LSC. LSC: all ^{14}C measurements were made using a liquid scintillation counter. LSC efficiency was computed for each sample using external standard channel ration values. HPLC Stationary phases: ODS-Hypersil (5 µm) or SAS-Hypersil (5 µm), both 100 x 4.6 mm) Mobile phase: sodium dihydrogen phosphate in distilled water (+ acetonitrile) either using a gradient system or isocratic; ammonium formate in distilled water plus acetonitrile following a gradient Flow rate: 0.5 and 1.0 ml/min. Detector: UV, 254 nm TLC: 2D-TLC, normal phase Plates: precoated with silica gel 60 F-254 at 0.25 mm thickness, two solvent systems MS: both, electron ionization (EI) and chemical ionization (CI) mass spectra were obtained

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products (2)

Annex Point IIA7.6.2.2

3.5	**Transformation products**	Yes; There was one component in the irradiated incubations that exceeded the 10% of the total applied dose. It was identified as CGA 294847 (denominated Ro 43-4756 in the report) and accounted for 12% at after 6 hours. Other degradation products were not identified but were all below 10% of the total applied dose: four of them between 5 and 10%, all others < 5%.	
3.5.1	Method of analysis for transformation products	TLC, HPLC and MS (see Point 3.4.9)	
		4 RESULTS	
4.1	**Screening test**	Not performed	
4.2	**Actinometer data**	The experimental spectrum of the SUNTEST equipment fits well with the spectrum of yearly-averaged, mid-day sunlight in the top millimetres of a natural aquatic system (sea level, latitude 40-50 °N). Small differences in the spectral region of higher absorbance of the a.i. were taken into account when the photolytic lifetime under sunlight is estimated using the quantum yield of the a.i.	X
4.3	**Controls**	Fenoxycarb under non-irradiated conditions in aqueous solution did not significantly degrade.	
4.4	**Photolysis data**		
4.4.1	Concentration values	1.0 mg/L	
4.4.2	Mass balance	The radiochemical balance during photodegradation study ranged from 99.0% to 106.9%.	
4.4.3	k^c_p	$2.852 \times 10^{-3} min^{-1}$ (non-sensitized samples, $r^2 = 0.987$) $7.455 \times 10^{-3} min^{-1}$ (sensitized samples, $r^2 = 0.914$)	
4.4.4	Kinetic order	First order kinetics	
4.4.5	k^c_p / k^a_p	n.a.	X
4.4.6	Reaction quantum yield (ϕ^c_E)	Using a p-nitroanisole-pyridine actinometer, the quantum yield of fenoxycarb was determined to be 6.5×10^{-2}.	
4.4.7	k_{pE}	n.a.	
4.4.8	Half-life ($t_{1/2E}$)	Non-sensitized samples: 243 min (4.1 hours) Sensitized samples: 93 min (1.5 h)	
4.5	**Specification of the transformation products**	n.a.	

Section A7.1.1.1.2
Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products (2)

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Photodegradation studies in water according to the guideline (see point 2.1).	
5.2	**Results and discussion**	The results are shown in Table A7_1_1_1_2-1. Fenoxycarb degraded under photolytic conditions with an experimental half-life of 4.1 hours and a rate constant of 2.852 x 10^{-3} min^{-1}. Under non-irradiated control or hydrolytic conditions at pH 7 fenoxycarb did not significantly degrade. In the presence of acetone as a sensitizer the degradation of fenoxycarb was faster with a half-life of 1.5 hours. Photolytic degradation led to formation of at least 11 components. Formation of ^{14}C-carbon dioxide was negligible with amounts of < 0.7% of the total dose after 6 hours.	X
5.2.1	k^c_p	See point 4.4.3	
5.2.2	K_{pE}	n.a.	X
5.2.3	ϕ^c_E	See point 4.4.6	
5.2.4	$t_{1/2E}$	For fenoxycarb, dissolved in the top millimetres of a natural aquatic system (situated at sea level, latitude 40 to 50 degrees North), and directly photolysed by the corresponding yearly-averaged, mid-day sunlight (corrected for reflection from the water surface) a photolytic lifetime of 6.3 hours (environmental half-life 4.5 hours, based on the quantum yield) was estimated.	X
5.3	**Conclusion**	The phototransformation of fenoxycarb is very fast with an experimental half-life of 4.1 hours. The rate of phototransformation is increased in the presence of a sensitizer as demonstrated by adding acetone to the photolysis solution. Thus, an acceleration of the investigated phototransformation can also be expected in natural water bodies, containing dissolved humic compounds as well known photosensitizers.	X
5.3.1	Reliability	Reliability indicator = 1	X
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2007/02/09

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

COMMENTS FROM ...

Date *Give date of comments submitted*

Materials and Methods *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Discuss if deviating from view of rapporteur member state

Results and discussion *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Reliability *Discuss if deviating from view of rapporteur member state*

Acceptability *Discuss if deviating from view of rapporteur member state*

Remarks

Table A7_1_1_1_2-1: Photolysis of fenoxycarb (1 mg/L) in buffer solution, unsensitized, in % of applied radioactivity

	Ethylacetate phase			Aqueous phase	Volatiles (CO_2)	Overall radio-recovery
Time [min]	Fenoxycarb	CGA 294847	Other photoproducts			
0	98.6	0	0.5	0.3	0.0	99.4
120	73.1	6.0	22.5	3.0	0.0	104.6
180	62.4	8.3	29.6	4.9	0.2	105.4
240	55.3	7.7	36.7	6..9	0.3	106.9
300	42.3*	11.6*	40.3*	7.0	0.4	101.6
360	35.2	12.3	47.3	8.0	0.7	103.5
360**	98.7	1.7		1.7	0.0	102.1

*Average of duplicate samples
** Dark control sample

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lebertz, H. (1990): CGA 114597, Ready Biodegradability (modified Sturm test) of RO-13-5223, Battelle Institut, Frankfurt, Germany, unpublished report No. BE-EA-25-89-01-STT-03. Study dates: October - December 1989. Issue date: 06 July, 1990. (Syngenta File No. /CGA1145970093).	
1.2	**Data protection**	No	
1.2.1	Data owner	Syngenta Crop Protection AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 301 B	
2.2	**GLP**	Yes (Battelle Institut, Frankfurt, Germany).	
2.3	**Deviations**	None	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Active Substance: ISO common name: fenoxycarb; Company Code: CGA 114597 Chemical name: [2-(4-phenoxy-phenoxy)-ethyl]-carbamic acid ethyl ester	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	Test substance: Technical grade fenoxycarb [redacted]	
3.1.4	Further relevant properties	Water solubility of fenoxycarb: 7.9 mg/l at 25 °C (Stulz, 1993)	x
3.1.5	Composition of Product	-	
3.1.6	TS inhibitory to microorganisms	Result of the respiration inhibition test with activated sludge according to OECD Guideline 209: EC_{50} > 100 mg/l (highest rate tested) (Grade, 2001)	x
3.1.7	Specific chemical analysis	-	
3.2	**Reference substance**	Yes, Sodium benzoate	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

3.2.1	Initial concentration of reference substance	20 mg DOC/L	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Polyvalent inoculum (sewage micro-organisms from a sewage plant in Frankfurt/M. Niederrad, Germany, working with predominantly domestic sewage)	
3.3.2	Test system	CO_2 evolved by the test material was trapped as $BaCO_3$	
3.3.3	Test conditions	Incubation was carried out at 21.0 to 22.5 °C.	
3.3.4	Method of preparation of test solution	A stock solution of the test substance as indicated in the Guideline could not be prepared because it was not sufficiently soluble in water, Special measures had to be introduced to achieve a correct final concentration of 10 and 20 mg/l.	
3.3.5	Initial TS concentration	10 and 20 mg/l	x
3.3.6	Duration of test	Since a plateau of the degradation curve was not reached after 28 days, the test was prolonged until day 41.	
3.3.7	Analytical parameter	The total amount of CO_2 produced by the test substance was calculated as a percentage of the total CO_2 that the test substance, based on its carbon content, could have theoretically produced (%TCO_2)	
3.3.8	Sampling	Day 4, 8, 14, 17, 22, 27, 31, 34, 38 and 41	
3.3.9	Intermediates/ degradation products	-	
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	Positive control with reference substance	
3.3.12	Statistics	The degree of biodegradation is expressed by the percentage of total CO_2 the test substance has generated in relation to the $ThCO_2$.	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	-	
4.1.2	Degradation	The biodegradation in this test seemed to be slow but constant. The results in detail are shown in Table A7_1_1_2_1- 1.	x
4.1.3	Other observations	No	
4.1.4	Degradation of TS in abiotic control	-	
4.1.5	Degradation of reference substance	The positive control Na-Benzoate was stopped after 141 hours because a degradation value of > 80% was reached indicating that the inoculum used for the test was active enough to achieve the required conditions.	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1

4.1.6	Intermediates/ degradation products	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was designed to assess the ready biodegradability of fenoxycarb and was conducted according to the OECD Guideline 301 B (CO_2 Evolution Test)	
5.2	**Results and discussion**	Calculated from the organic carbon content of the test substance and the measured CO_2 generation, 78% of the theoretical CO_2 ($ThCO_2$) has been generated by the test substance within 36 days in the case of the 10 mg/l culture. The test system had generated 24% of the theoretical CO_2 within 43 days in the case of the 20 mg/l culture. A plateau was not reached.	
5.3	**Conclusion**	Since the criteria for "ready biodegradability" (pass level of 60% ThOD within 28 days; 10-day window) are not fulfilled, fenoxycarb has to be characterised as not readily biodegradable. However, based on these results fenoxycarb is classified as "inherently ultimately biodegradable" according to the definitions given by the OECD guidelines for testing inherent biodegradability (OECD Guideline for Testing of Chemicals; Proposal for Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, Part 1: Principles and Strategies related to the Testing of Degradation of Organic Chemicals; OECD, April 2005).	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	Some details are missing.	

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2008/02/20

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks

[redacted]

COMMENTS FROM ...

Date *Give date of comments submitted*

Materials and Methods *Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Discuss if deviating from view of rapporteur member state

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_1_1_2_1- 1: Results of testing biodegradability of fenoxycarb

	Test solution with 10 mg/l		Test solution with 20 mg/l	
Time [days]	CO_2 generated [mg]	TCO_2 [%] * (= % biodegradation)	CO_2 generated [mg]	TCO_2 [%] * (= % biodegradation)
4	0	0	0	0
8	1.17	1.3	1.17	0.7
14	9.36	10.8	4.02	2.3
17	22.35	25.7	6.28	3.6
22	39.85	45.9	9.64	5.5
27	50.68	58.3	13.03	7.5
31	55.18	63.5	13.20	7.6
34	62.17	71.5	20.52	11.8
36	68.03	78.3	-	-
38	-	-	33.74	19.4
41	-	-	38.49	22.1
43	-	-	42.14	24.1

* TCO_2: percentage of total CO_2 the test substance has generated in relation to the $ThCO_2$

Section 7.1.1.2 Annex Point IIA, VII 7.6.2.1	Inherent biodegradability	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	

Section 7.1.1.2.2 Annex Point IIA, VII 7.6.2.1	Inherent biodegradability
	[redacted]
Undertaking of intended data submission [ ]	–

Evaluation by Competent Authorities	
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008-02-22
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
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Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 7.1.2.1.1 Annex Point IIIA 12.2	Aerobic biodegradation with respect to biological sewage treatment	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X].**	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
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	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	08/02/07	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
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Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.2.2.2 Water/sediment degradation study (1)

Annex Point IIIA XII2.1 **Microcosm study**

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kennedy, J.H., Reed, C.W. and Hosmer, A.J. (1995): Assessment of the potential biological effects of fenoxycarb exposures on aquatic ecosystems as measured in an outdoor fiberglass tank system (microcosms). Water Research Field Station, University of North Texas, Denton, USA and ABC Laboratories, Inc., Columbia, USA, unpublished report No. CMP3. Issue date: 19 June, 1995 (Syngenta File No. CGA114597/0555)	
1.2	**Data protection**	No	
1.2.1	Data owner	Syngenta Crop Protection AG	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; U.S. EPA FIFRA Guideline No. 72-7(a).	
2.2	**GLP**	Yes (certified laboratories).	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
		The study was conducted using 24 fibreglass tanks having each a volume of 10 m^3 (1.7 m deep, 3 m diameter, depth of water body 1.5 m, sediment layer 15 cm) located at the University of North Texas Water Research Field Station. The water/sediment characteristics are shown in Table A7_1_2_2_2-1. There were a total of eight treatments with three replicate tanks per treatment. The uses of fenoxycarb indicate that off-target movement would result from run-off and spray drift. Fenoxycarb was therefore applied to the microcosms as an adsorbed phase (125.5 g of sediment per tank) in the form of slurry and as a dissolved phase in the form of an acetone/water solution. One replicate served as a control (D0), which received an application of acetone/water solutions and soil slurries. The remaining seven replicates (D1 - D7) were dosed at 0.014, 0.041, 0.123, 0.37, 1.11, 3.33, 10.0 µg/l. The three lower doses received single applications, the higher doses (D4 to D7) received a second application after four weeks. The first application to the tanks was made on May 17, 1993. The dosing was at concentrations, which approximate and/ or encompass expected environmental fenoxycarb exposures. Exposure concentrations for the study were estimated by using US environmental fate and transport models. Model scenarios simulated application rates of 0.14 kg as/ha at the end of April and at the beginning of June in orchards (apple and pear). Pesticide runoff was simulated using the Pesticide Root Zone Model (PRZM-2.2 and PRZM-2.3) for a 10-ha	

field. Mass loading to the pond by spray drift was included in the simulations as 5 percent of the maximum application rate of 0.14 kg as/ha. The loadings were predicted for the U.S. Environmental Protection Agency "standard pond" scenario, which was modelled using the Exposure Modelling System, version 2.95 (EXAMSII). US EPA's standard pond is configured as having a 1-ha surface area and 2-m depth. In Table A7_1_2_2_2-2, the theoretical direct overspray application rates (g as/ha) for a 150 cm and a 30 cm water body are correlated with the fenoxycarb concentrations in the test tanks.

Technical grade fenoxycarb (97.8 % as) was used to prepare application solutions. Results of the analyses of application stock solutions, application dosing solutions, and microcosm water samples immediately after treatment indicated that all tanks were dosed with the proper amount of fenoxycarb (99 ± 14% of nominal). To aid the determination of the dissipation rate of fenoxycarb in the microcosms at different concentrations, water and hydrosoil samples were collected at selected time points. Sediment samples corresponded to 2.5 cm layer cores. ABC Laboratories (Columbia, MO) performed analysis of test compound in water and hydrosoil.

4 RESULTS

Results were presented of an outdoor microcosm study designed to examine the chemical fate and potential biological effects of fenoxycarb in aquatic ecosystems.

Application of fenoxycarb appeared to have no effect on the physico-chemical parameters that were recorded, such as temperature, dissolved oxygen, turbidity, pH, total alkalinity and total water hardness. However, it should be noted that the actual concentration of fenoxycarb in those replicates which received 2 applications (D4-D7) may be higher than the nominal concentrations.

The lower limit of quantification (LOQ) for parent fenoxycarb was 0.001 µg /l in the microcosm water. In the sediment the LOQ for fenoxycarb was 1.0 µg /kg sediment.

The overall recovery of fenoxycarb in water was 90% with a 13% standard deviation. Recovery of fenoxycarb from laboratory spiked hydrosoil samples at 1.0 µg/kg gave an average recovery of 104% with an 11% standard deviation, indicating the method was working properly. Data from field spiked hydrosoil samples gave an overall average 49 ± 25% recovery. The discrepancy could not be explained, but was demonstrated not to have been caused by instability of fenoxycarb during freezer storage or by inefficiency of the extraction method employed.

In Table A7_1_2_2_2-3, the results for fenoxycarb analysed in microcosm water are shown and expressed in concentrations of µg/l. The concentration of fenoxycarb in the aquatic water bodies under natural conditions decreased rapidly. Calculated dissipation half-lives DT_{50} in water of the individual tanks at various concentrations after single and duplicate applications are shown in Table A7_1_2_2_2-4.

Section A7.1.2.2.2 **Water/sediment degradation study (1)**

Annex Point IIIA XII2.1 **Microcosm study**

Considering all dose levels from 0.014 to 10.0 µg/l and single as well as second treatments, a representative half-life for fenoxycarb was determined to be DT_{50} = 12.2 hours in the microcosm water (Table A7_1_2_2_2-5).

The rate of dissipation of fenoxycarb in the water was dose specific as shown in Figure A7_1_2_2_2-1.

With only two exceptions, 1.7 µg/kg for tank 55 (Dose 7) on study day one, and 1.3 µg/kg for tank 63 (Dose 7) on study day 14, fenoxycarb was not detected in the sediment samples in this study. Due to the low dosing concentrations used and the multiple dissipation processes including photolysis, microbial degradation and adsorption in the microcosm, fenoxycarb apparently was not present in the sediment in appreciable amounts and the maximum concentration levels reached were close to the LOQ of 1 µg/kg making further analysis not meaningful.

5 CONCLUSION

5.1 Conclusion

Based on the residue levels in the microcosm water body, a (median) dissipation half-life of 12.2 hours (= 0.5 days) was calculated for fenoxycarb (quickly elimination from the water phase).

The Limit of Quantification (LOQ) for fenoxycarb in sediment was 1 µg/kg. Due to the low residue levels in this study the LOQ was slightly exceeded only twice in the hydrosoils of two different tanks at different sampling intervals. Therefore the results show that fenoxycarb levels in the sediment resulting from comparable dose levels are minimal, but do not allow for any dissipation calculation.

5.1.1 Reliability 1

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/02/18
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

A7_1_2_2_2-1: Water/sediment characteristics of microcosm tanks

Sediment characteristics:	
sand [%]:	60 - 72
silt [%]:	6 - 20
clay [%]:	16 - 26
pH	8.3 - 8.5
organic carbon [%]:	0.3 - 0.4
Texture	Sandy loam - sandy clay loam
Water characteristics	
pH	8.6 - 10
Calcium hardness [mg $CaCO_3$/l]	24 - 78
DOC [mg/l]	8.5 - 16
Temperature [°C]	19 - 29

Table A7_1_2_2_2-2: Theoretical fenoxycarb direct overspray rates corresponding to the test concentrations of the microcosm study

Dose level	Test conc. in tanks	Field application rate direct overspray [g as/ha]	
	[µg as/l]	150 cm water depth	30 cm water depth
D1	0.014	0.21	0.042
D2	0.041	0.615	0.123
D3	0.123	1.845	0.369
D4	0.37	5.55	1.11
D5	1.11	16.65	3.33
D6	3.33	49.95	9.99
D7	10	150	30