	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	

9. NEUROTOXICITY

98/8 Doc IIIA 6.9.1 section No.	Acute Neurotoxicity Section A6.9.1 Acute neurotoxicity in rat (gavage)	Official use only
91/414 Annex IIA 5.7 Point addressed		

Title:	Acute Neurotoxicity Study In Rats.	
Lab Report Number:	AR7547/Regulatory/Report	
Authors:	[redacted] (2006a)	
Test Substance:	Abamectin technical (MK936)	
Species:	Rat	
Guidelines:	OECD 424 (1997); OPPTS 870.6200 (1998) JMAFF test Guideline 2-1-12	
Date of Report:	25 August 2006	
Published:	No	
GLP:	Yes	

STUDY 1

Characteristics

Reference/notifier	:	[redacted] 2006a	Exposure	:	single oral administration (gavage)
Type of study	:	acute neurotoxicity	Doses	:	0, 0.5, 1.5, 6 mg/kg bw
Year of execution	:	2006	Vehicle	:	sesame seed oil
Test substance	:	Abamectin technical (MK936), purity [redacted]. Batch [redacted]	GLP statement	:	Yes
Route	:	oral (gavage)	Guideline	:	OECD 424
Species	:	Rat (Alpk: APfSD [Wistar-derived])	Acceptability	:	acceptable
Group size	:	10/sex/dose	$NOAEL_{neurotoxicity}$	:	0.5 mg/kg bw

Study design

Groups of Alpk: APfSD (Wistar-derived) rats (10/sex/dose) received a single oral (gavage) dose of

abamectin at 0, 0.5, 1.5 or 6 mg/kg bw. Vehicle was sesame seed oil. Detailed clinical examinations were performed daily. Body weight was measured prior to treatment and at days 1 (6-7h after dosing, assumed time of plasma peak concentration), 8 and 15. Food consumption per cage of 5 rats was calculated at weekly intervals. The rats were tested in a functional operational battery (FOB) and for locomotor activity prior to treatment and at days 1 (6-7 h after dosing), 8 and 15. At day 15 the animals were killed. Five rats/sex/dose were macroscopically examined, brains were weighed and the eyes and various nervous tissues were histologically examined.

Results

Results from a single dose neurotoxicity study with abamectin in the rat.

Dose (mg/kg bw)	**0**		**0.5**		**1.5**		**6.0**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No toxicologically relevant effects								
Clinical signs	see functional observational battery								
Body weight	No toxicologically relevant effects								
Food consumption	No toxicologically relevant effects								
Functional observational battery[A] **day 1** - reduced splay reflex - tiptoe gait - splayed gait					2/10	3/10	7/10	8/10[B] 1/10 1/10	dr
Motor/locomotor activity measurements day 1 0-15 min								-48%*	
Gross pathology	No toxicologically relevant effects								
Brain weight								- 5% *	
Neuropathology									
- macroscopy	No toxicologically relevant effects								
- microscopy	No toxicologically relevant effects								

* Statistically significant

[A] Number of animals affected/number of animals tested.

[B] In the 6 mg/kg bw group 3 and 1 females also displayed reduced splay reflex at days 2 and 3, respectively.

Acceptability

The study is considered acceptable

Conclusions

Based on the reduced splay reflex, observed on day 1 (6-7 h after dosing) at doses of 1.5 and 6 mg/kg bw, the NOAEL is 0.5 mg/kg bw.

98/8 Doc IIIA 6.9.2 section No.	Repeated dose neurotoxicity Section A6.9.2 Neurotoxicity, 90 day rat	Official use only
91/414 Annex Point addressed	IIA 5.7	

Title:	Abamectin Technical (Mk936) 90 Day Combined Oral Toxicity And Neurotoxicity Study In Rats	
Lab Report Number:	PR1325-REG	
Authors:	[redacted] (2006b)	
Test Substance:	Abamectin technical (MK936)	
Species:	Rat	
Guidelines:	OECD 408, OECD 424; OPPTS 870.6200 (1998); OPPTS 870.3100 (1998); JMAFF Test Guideline 2-1-12.	
Date of Report:	25 August 2006	
Published:	No	
GLP:	Yes	

STUDY 2

Characteristics

Reference/notifier	:	[redacted] 2006b	Exposure	:	90 days (gavage)
Type of study	:	semi-chronic toxicity/neurotoxicity	Doses	:	0, 0.4, 1.6, 4 mg/kg bw
Year of execution	:	2006	Vehicle	:	sesame seed oil
Test substance	:	Abamectin technical (MK936), purity [redacted]. Batch [redacted]	GLP statement	:	Yes
Route	:	oral (gavage)	Guideline	:	OECD 408, OECD 424
Species	:	Rat (Alpk: APfSD [Wistar-derived])	Acceptability	:	acceptable
Group size	:	16/sex/dose	$NOAEL_{neurotoxicity}$	:	1.6 mg/kg bw/day

Study design

In a combined semi-chronic toxicity/neurotoxicity study groups of Alpk: APfSD (Wistar-derived) rats

(16/sex/dose) received daily oral (gavage) doses of abamectin at 0, 0.4, 1.6 or 4 mg/kg bw for 90 days. Vehicle was sesame seed oil. Cage-side clinical examinations were performed daily. Body weight was measured weekly. Food consumption per cage of 5 rats was calculated at weekly intervals. All rats were tested in a FOB and for locomotor activity at week 14. In addition, at least 10 rats/sex/dose were tested in the FOB and for locomotor activity prior to treatment and at weeks 2, 5 and 9. Ophthalmoscopy was performed on all animals prior to testing, and on control and mid-dose animals during week 13. During week 13 urine samples were collected for urinalysis. At termination in week 14 blood was collected for haematology and clinical chemistry. Five rats/sex/dose were killed by perfusion fixation and subsequently brain weight was recorded and nervous tissue (including eye) was histologically examined. All other animals (including any killed prematurely) were killed by over-exposure to halothane and exsanguination, and subsequently macroscopically examined. For rats killed by over-exposure to halothane and exsanguinations at termination an extensive range of organs were weighed and subsequently histologically examined.

Results

Results from a repeated dose neurotoxicity study with abamectin in the rat.

Dose (mg/kg bw/day)	**0**		**0.4**		**1.6**		**4**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality[A,B]							16/16	16/16	
Clinical signs	no toxicologically relevant effect						is[C]		
Body weight	no toxicologically relevant effect						ds[D]		
Food consumption	no toxicologically relevant effect						ntre[E]		
Ophthalmoscopy			ND		ntre		ND	ND	
Functional observational battery	no toxicologically relevant effect						is[C]		
Motor/locomotor activity measurements [F]	no toxicologically relevant effect								
Haematology	no toxicologically relevant effect						ND		
Clinical chemistry	no toxicologically relevant effect						ND		
Urinalysis	no toxicologically relevant effect						ND		
Organ weight	no toxicologically relevant effect						ND		
Brain weight	no toxicologically relevant effect						ND		
Macroscopy [A] - ulceration and red spots of non-glandular stomach	no toxicologically relevant effect						1/16[G]	1/16[G]	
Microscopy [A] - inflammation and focal ulceration of the stomach	no toxicologically relevant effect						4/16[G]	3/16[G]	

Neuropathology			
- macroscopy	no toxicologically relevant effect	ND	
- microscopy	no toxicologically relevant effect	ND	

is: increased significantly. ds: decreased significantly. ntre: no toxicologically relevant effect. ND: no data

[A] Number of animals affected/number of animals tested.

[B] During week 7 the animals of the high-dose group showed a sudden and progressive body weight loss and increased incidence of adverse clinical signs (see B,C). These animals were killed for humane reasons. In both the low- and mid-dose group one female died due to dosing errors. One female of the mid-dose group fell onto the floor during handling, was convulsing and immediately humanely killed.

[C] During cage-side observations and FOB testing clinical signs were seen in animals of the high-dose group at relatively low incidence from week 2 onwards. In these animals, a marked increase in adverse clinical signs (shaking, tiptoe gait, reduced righting reflex, reduced stability, reduced splay reflex, hunched posture, "pinched-in" sides, subdued behaviour, irregular breathing, decreased activity, stains around the mouth or nose, upward spinal curvature) was observed in week 7.

[D] Slight (up to 7%) but statistically significant reductions in body weights of high-dose females were observed during weeks 2-5. All high-dose animals showed body weight loss during week 7.

[E] No effects on food consumption were observed up to week 6. No data on food consumption in week 7 are available.

[F] High dose animals tested at weeks 2 and 5 only.

[G] High dose animals were killed at weeks 7/8.

Acceptability

The study is considered acceptable.

Conclusions

Based on the adverse clinical signs, body weight loss and macroscopic and histological changes in the stomach, observed at 4 mg/kg bw, the NOAEL is 1.6 mg/kg bw/day.

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*5 June 2008; updated January 2009*
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	

Delayed neurotoxicity

Note of the notifier:

Justification for not providing studies on delayed neurotoxicity: [redacted]

Conclusion: [redacted]

[redacted]

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12 November 2007; updated January 2009	
Evaluation of applicant's justification		
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
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		

10. MECHANISTIC DATA

Mechanistic studies have not been performed (see justification below), but an important aspect to consider in the risk assessment of abamectin is the role of p-glycoprotein polymorphism. The text below is copied from the revised addendum (Febr. 2008) for Council Directive 91/414/EC concerning the placing of Plant Protection Products on the market.

The applicant submitted a summary of the literature on the relevance of MDR-1 polymorphism for humans which has recently been published:
Macdonald, N. & Gledhill, A. (2007). Potential impact of ABCB1 (p-glycoprotein) polymorphisms on avermectin toxicity in humans. Arch. Tox. 81 (8): 553-563.
Below, a summary of the submitted publication (see publication for the cited references) and the RMS's concluding remarks will be presented. The summary of the publication is rather extensive, because this is considered necessary for a good understanding of the role of p-glycoprotein polymorphism and its relevance for the risk assessment of abamectin.

Summary of the publication by Macdonald & Gledhill (2007)

Function and structure

The ATP binding cassette (ABC) transporters are a superfamily of large, membrane bound proteins that mediate the active trafficking of molecules across cellular membranes in an ATP dependent manner, often against considerable concentration gradients. Several ABC transporter proteins exhibit xenobiotic cellular efflux activity. Perhaps the best characterised of these is p-Glycoprotein (pgp), which has been shown to transport a structurally diverse range of chemicals, including the anthelminthic drug ivermectin and structurally related avermectin pesticides. The pgp gene is known as ABCB1, but also as MDR1 (multi drug resistance) based on its over expression in drug resistant tumours. In humans pgp is expressed in polarized cells in a wide range of tissues, and in each case it functions as a component of a barrier protecting one compartment from the contents of another, pumping potentially toxic compounds away from the sensitive compartment. For example, brain capillary epithelial cells of the blood brain barrier (BBB) are linked by tight junctions, meaning compounds may only pass across the BBB by diffusion through these cells. pgp transports substrate chemicals back into the blood, limiting diffusion across the BBB and protecting the brain. Similarly in the placenta pgp transports xenobiotics back into the maternal blood, thus protecting the fetus. pgp is also expressed in testis, hepatocytes, kidney proximal tubules, and intestinal epithelial cells. Because of this tissue specific expression and cellular polarisation pgp contributes to three layers of protection: limiting absorption of xenobiotics from the gut, removing xenobiotics from the blood by excretion via bile and urine, and protecting the fetus, and vulnerable organs such as the brain and testis

through its role in barrier epithelia.

P-glycoprotein genes are found in all animals. Humans and dogs have a single gene (capitalised when referring to humans, i.e. ABCB1/MDR1, lowercase for animals, e.g. abcb1/mdr1 in dogs) while rodents have two (abcb1a/mdr1a and abcb1b/mdr1b; each have similar substrate specificity to their human homologue, and between them they cover all the tissues in which pgp is expressed in humans). These genes are highly conserved in mammals and human ABCB1 exhibits 87, 90, and 87% amino acid homology with rat and mouse abcb1a, and dog abcb1, respectively.

Role of P-glycoprotein in limiting avermectin toxicity

Avermectins are a group compounds derived from the macrocyclic lactone avermectin b1, which was initially isolated from *Streptomyces avermitilus*. They are widely used as treatments for parasitic infestations in humans and animals, as well as agricultural insecticides and acaricides. In all uses of avermectins the mode of action is avermectin binding to invertebrate neuronal gamma-aminobutyric acid (GABA) receptors and GABA gated ion channels, resulting in net cellular influx of chloride ions into neurones, leading to paralysis and eventual death. Typically humans given ivermectin as an anthelminthic receive doses of between 0.05 and 0.2 mg/kg. Exposure of workers using avermectin pesticides varies depending on the use/formulation of the product, but has been estimated to be several orders of magnitude below the clinical dose.

Although avermectins can also bind to mammalian GABA receptors and GABA gated ion channels, albeit weakly, neurotoxicity is negated by poor avermectin penetration of the mammalian blood brain barrier in all but extreme cases of avermectin self poisoning.

C57BL/6 derived abcb1a knockout mice and some CF-1 mice were found to exhibit ivermectin sensitivity. CF-1 mouse ivermectin sensitivity exhibited classic Mendelian inheritance patterns, and has since been shown to be due to retroviral insert in exon 23 of the abcb1a gene in some CF-1 mice. This results in total absence of properly transcribed, functional pgp in CF-1 mice homozygous for the disrupted form of the gene. In dog breeds related to the collie a four base pair deletion has occurred in abcb1 resulting in a loss of pgp function leading to an increase in sensitivity to ivermectin neurotoxicity
in dogs homozygous for the mutated version of the gene.

In both the CF-1 and the C57BL/6 mdr1a null mice models oral ivermectin dosing results in plasma ivermectin concentrations 2.5-fold to 3.3-fold higher in pgp null mice than in the wild type mice 24 h after dosing. Lack of pgp dependent efflux at the BBB also allows vastly increased brain penetration of avermectins. Brain ivermectin concentrations 24 h post dosing are between 33-fold and 87-fold higher in pgp null mice compared to wild type mice. Studies in our laboratory have shown similar results for two other avermectins, emamectin and abamectin, which are used predominantly as pesticides. Homozygous

pgp null (abcb1a –/–) mice show increased susceptibility to 0.2 mg/kg oral abamectin, while heterozygous (abcb1a +/–) mice and wild type mice (abcb1a +/+) are insensitive to up to 2.5 mg/kg abamectin. LD50 data indicates that at very high doses heterozygous mice are slightly more abamectin sensitive than homozygous wild type mice (–/+ LD50 = 14 mg/kg, +/+ LD50 = 30 mg/kg, –/– LD50 = 0.3 mg/kg). Thus although heterozygous mice express less brain pgp, a single copy of a functional abcb1a gene is sufficient for adequate pgp functionality in the mouse BBB at doses of avermectins used in the clinic (0.2 mg/kg), or resulting from worker pesticide exposure.

Where placental pgp activity is compromised avermectins can also exhibit developmental toxicity. In pgp null mice foetal avermectin exposure is associated with increased incidence of cleft palate. The placenta is a foetal tissue, and as such avermectin developmental toxicity is dependent on the abcb1a status of the fetus. CF-1 abcb1a –/– fetuses of mothers treated with abamectin have significantly higher concentrations of abamectin in their plasma than their abcb1a +/+ and +/– littermates. Similarly when CF-1 dams were dosed with 1.5 mg/kg abamectin, all abab1a –/– fetuses developed cleft palates, while none of their abcb1a +/+ littermates and only 30% of their +/– littermates developed cleft palates.

Significant neonatal ivermectin neurotoxicity is seen in rat pups through a combination of ivermectin exposure of the offspring of ivermectin dosed rat dams via the dams' milk, and lack of pgp expression in the neonatal rat brain. However, this is not thought to be relevant to human risk assessment as brain pgp expression starts early in human development, having been detected in human foetal brain microvessels as early as week eight of pregnancy.

P-glycoprotein human polymorphisms and pgp haplotypes

Naturally occurring mutations that lead to non-functional pgp have been found in both the CF-1 strain of mice and dog breeds closely related to the collie. Millions of humans have received ivermectin as an anthelminthic treatment for river blindness without reports of major adverse neurological effects, although arguably adverse effect reporting may be less robust in the areas of the world where river blindness occurs. In addition, cumulatively more than 4,000 human volunteers have been genotyped for ABCB1 [although often only for known single nucleotide polymorphisms (SNPs)] without reports of major rearrangements of the ABCB1 gene similar to those in the CF-1 mouse and collie dog. Taken together this may indicate that individuals with significantly compromised pgp functionality analogous to that seen in the CF-1 pgp –/– mouse are rare.

More than 50 naturally occurring SNPs have been identifed in the human ABCB1 gene. The vast majority are silent, i.e. they either do not occur in the coding region of the gene, or due to the inherent redundancy of codon usage they do not alter the amino acid sequence of the protein. As has been extensively reviewed elsewhere there are numerous conflicting reports of the effects of individual ABCB1 SNPs on pgp

expression and function in various tissues. Also the submitted publication gives an extensive overview of publications on the effects of individual ABCB1 SNPs. The conclusion is that there is no clear pattern of clinical effect of individual SNPs on pgp mediated efflux.
It is therefore suggested that combinations of human SNPs (haplotype) may be important in determining phenotype. An overview of the literature on pgp haplotypes is presented in the publication. This includes studies in which human BBB pgp function has been measured directly. Although various human ABCB1 haplotypes and/or SNPs have been reported to alter pgp function in relation to gut absorption, at present there is no conclusive data indicating that any of the known common haplotypes, including homozygosity for the most common minority haplotype, result in a significant loss of BBB pgp functionality. This would tend to indicate that the CF-1 mdr1a –/– mouse strain, which completely lacks pgp BBB functionality, is not a representative model for assessing risk in humans homozygous for any of the known haplotypes.

Population distribution of pgp haplotypes

Populations with different ethnicities are known to have different distributions of the various pgp haplotypes. Forty-eight and 79% of ABCB1 haplotypes found in the African American and Caucasian populations, respectively, produce a pgp identical to the reference amino acid sequence. Of the remainder, 38% of African American and 7.5% of Caucasian ABCB1 genes represent a haplotype which contains only one nonsynonymous SNP. Data from *in vivo* studies indicates that alleles in these two categories both produce pgp that is functional in the BBB. Given the sampled population frequencies of the commonest pgp haplotypes, and the fact that at clinically relevant doses a single functional copy of abcb1a is sufficient to prevent avermectin neurotoxicity in the CF-1 mouse, it is possible to calculate the proportion of the human populations that are likely to exhibit normal pgp BBB functionality (see publication for more details). >98% of people in African American and Caucasian populations will carry at least one copy of an ABCB1 haplotype that is already known to encode a pgp that is functional in the BBB and will therefore not be at risk of toxicity from the concentrations of avermectins to which humans are typically exposed. Between 1 and 2% of the population would thus carry only haplotypes with unconfirmed BBB functionality. Each individual "unconfirmed BBB functionality" haplotype is relatively rare within the population, often only having been identified in a single heterozygous individual, with each "rare" haplotype having an allelic frequency of less than 1%. As such, individuals that are homozygous for any one of the haplotypes with unconfirmed BBB functionality would be very rare within the population (<0.01%). If any of these rare haplotypes exhibited significantly compromised BBB pgp functionality it is likely that individuals homozygous for that haplotype, and thus having compromised BBB pgp function, would be extremely rare.

Conclusions

pgp dependent xenobiotic effux in the blood brain barrier and placental mother/fetus barrier play an important role in attenuating the known neurotoxicity of avermectins and the developmental toxicity of ivermectin and abamectin. There is currently no evidence for the existence of mutations of the ABCB1 gene in the human population that result in a loss of function analogous to that seen in the CF-1 mouse and collie dog. Although there are numerous reports for and against the proposition that some ABCB1 SNPs and/or haplotypes exhibit reduced pgp expression and function, there are no consistent data indicating that known SNPs or haplotypes have an adverse effect on pgp function in the BBB or placenta. Where human BBB pgp function has been measured directly the most common haplotypes were found to have equal functionality. Since heterozygous pgp +/– mice and dogs do not exhibit ivermectin neurotoxicity at clinically relevant doses it is likely that humans carrying at least one functional copy of ABCB1 will not be more susceptible to avermectin toxicity at clinically relevant doses or at the low exposure levels resulting from pesticide use. Calculations using allelic frequencies of known haplotypes indicate that homozygosity for any as yet uncharacterised haplotypes with severely reduced BBB functionality is likely to be very rare in human populations.

RMS's concluding remarks

[redacted]

[redacted]

Justification below not/partially reported in DAR

98/8 Doc IIIA section No.	6.10	Mechanistic studies	Official use only
91/414 Annex Point addressed	Not presented in IIA		

Section 6.10 Annex Point IIA 6.10	Mechanistic study	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the	

Section 6.10 Annex Point IIA 6.10	Mechanistic study	Official use only
	comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12 November 2007	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
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		

11. STUDIES ON OTHER ROUTES OF ADMINISTRATION

98/8 Doc IIIA 6.11 section No.	Other routes of administration	Official use only
91/414 Annex Point addressed	Not applicable	

Section 6.11 Annex Point IIA 6.11	Other routes of administration	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12 November 2007	

Section 6.11 **Annex Point IIA 6.11**	**Other routes of administration**	**Official use only**
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
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		

12. MEDICAL DATA IN ANONYMOUS FORM

98/8 Doc IIIA 6.12.1 section No.	Medical surveillance data on manufacturing plant personnel if available.	Official use only
91/414 Annex II Point addressed 5.9.1	Medical Surveillance data on manufacturing plant personnel	
	Manufacturing employees are medically examined by company physicians at the beginning of their employment and then routinely once a year. In Switzerland, routine medical examinations according to the criteria of the Swiss Accident Insurance Institution (SUVA) include: Anamnesis Physical examination Blood analysis: haemoglobin, erythrocytes, leukocytes, thrombocytes, complete blood count, blood sedimentation rate, blood sugar, blood pressure, cholesterol, triglycerides, ALAT, ASAT, alkaline phosphatase, bilirubin, creatinine, uric acid Urine analysis	

98/8 Doc IIIA section No.	6.12.2/01	Human case reports (medical surveillance data)	Official use only
91/414 Annex Point addressed	II 5.9.2	Direct observation e.g. clinical cases, poisoning incidents if available	

Medical surveillance on manufacturing

- Data from persons exposed in manufacturing at the [redacted]

  In the observation period 1998 to 2000 the annual production volume (formulation) was in the range [redacted]. The manufacturing was performed in about [redacted].

  No adverse health effects have been reported which could be related to abamectin.

- Data from persons exposed in manufacturing at the [redacted]

  In the observation period 1999 to 2000 the production volume (formulation) was in the range of [redacted]. The manufacturing was performed in [redacted]

  No adverse health effects have been reported which could be related to abamectin.

98/8 Doc IIIA section No.	6.12.2/02	Reports on direct observations, e.g. clinical cases and poisoning incidents	Official use only
91/414 Annex Point addressed	II 5.9.2 / 02	Reports on direct observations, e.g. clinical cases and poisoning incidents	

Title:	Agricultural Avermectins: An Uncommon But Potentially Fatal Cause of Pesticide Poisoning	
Lab Report Number:	Ann Emerg Med (34) 51-57	
Authors:	Chung, K. *et al.*	
Test Substance:	Agricultural avermectin	
Species:	Human	
Guidelines:	Not applicable	
Date of Report:	Not applicable	
Published:	Yes	
GLP:	No	

Wu, M., C. Yang and K. Chung (1999)

Inquiries concerning agricultural avermectin poisoning received by a poison center in Taiwan from September 1993 through December 1997 were identified. The demographic and clinical data of 18 patients (14 males and 4 females) exposed to AgriMek (2% wt/wt abamectin) are presented ranging in age from 15 to 83 years. Reasons for exposure included suicide attempts in 14 patients, occupational exposure in 3 patients and accidental exposure in 1 patient. The route of exposure was oral in 14 patients, inhalation in 2 patients and dermal contact in 2 patients.

The most common reported toxic effects involved the central nervous system (CNS, 11 patients), gastrointestinal system (GI, 8 patients) or cardiovascular system (6 patients). Based on their clinical severity, these 18 patients can be divided into 3 groups (see table).

Symptoms of poisoning after oral ingestion of Agri-Mek (a.i. = abamectin)

Group	Mortality	Average a.i. Dose [mg/kg]	Clinical Features*	Treatment
Severe	1/6	114.9 (range 38.5-227.3)	**CNS-effects and hypotension:** Aspiration pneumonia/respiratory failure, coma, fever, hypotension, leukocytosis, tachycardia, salivation, vomiting, rhabdomyolysis, metabolic acidosis, tachypnea, hypokalemia, hypocalcemia, hypothermia, pulmonary edema, drowsiness	Intensive Care
Mild	0/8	25.1** (range 4.2-67.0)	**Mild and shortlasting CNS and GI effects:** Diarrhea, nausea/vomiting, drowsiness, salivation, weakness	Supportive measures and observation
Asymptomatic	0/4	21.4 (range 12.5-41.7)	-	Observation

* some clinical features of the GI tract may probably be related to coingested substances (alcohol, methomyl, pyrethroids and methamidophos).

** the calculation of this average dose is based on 3 reported doses only. In 5 cases (3 occupational exposure, one accidental exposure and one attempt of suicide) no amount of ingested Agri-Mek was reported

Conclusion: Ingestion of a large dose of avermectin may be associated with life-threatening coma, hypotension and subsequent aspiration. Follow up for up to 6 months showed a quick and complication-free recovery of all patients, except one patient who died as a result of multiple organ failure (dose 88.1 mg/kg bw). The therapy for abamectin poisoning is mainly symptomatic and supportive.

SYNGENTA CONCLUSIONS

Conclusions:	Humans show a low susceptibility towards the toxicity of Abamectin. The therapy for Abamectin poisoning is mainly symptomatic and supportive. Despite the lack of specific therapy, the follow-up of severely poisoned patients showed a rather uneventful recovery. Therefore prognosis is good unless complication from severe uncontrolled aspiration occurs. The toxicity of co-ingested compounds (formulation agents, other active ingredients and alcohol) has to be taken into account.	

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA section No.	6.12.2/03	Health records, both from industry and other available sources	Official use only
91/414 Annex Point addressed	II 5.9.2/01		

Title:	MK-0936 (Avomec) - reports of accidental injections in man	
Lab Report Number:	Not applicable	
Authors:	Jeremy, D. (1985), Scott, P. (1986):	
Test Substance:	Avomec (a 1% w/w injectable formulation of Abamectin for veterinary use)	
Species:	Human	
Guidelines:	Not applicable	
Date of Report:	5 November 1985, 6 November 1985 and 4 September 1986.	
Published:	No	
GLP:	No	

Jeremy, D. (1985) and Scott, P. (1986)

Three reports of accidental injection of Avomec (a 1% w/w injectable formulation of abamectin for veterinary use) with approximately 30, 40 and 50 mg abamectin have been made, originating in Australia. None of the 3 subjects displayed adverse clinical signs other than a localised reaction at the puncture site.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA section No.	**6.12.4**	**Epidemiological studies on the general population, if available.**	**Official use only**
91/414 Annex Point addressed	**II 5.9.3**	Observations on exposure of the general population and epidemiological studies if appropriate	

Observations on exposure of the general population

No epidemiological study has been performed by the company. No reports from the open medical literature are on record.

98/8 Doc IIIA section No.	6.12.5	Human case report (diagnosis of poisoning including specific signs of poisoning and clinical tests, if available)	Official use only
91/414 Annex Point addressed	II 5.9.4	Diagnosis of poisoning (determination of active substance, metabolites) including specific signs of poisoning and clinical tests, if available	

Clinical signs and symptoms of poisoning and details of clinical tests

There are no specific signs of poisoning with abamectin.

Available human data from suicide attempts show that typical clinical signs of abamectin toxicity in animal studies, like tremors and convulsions, do not occur in humans. At ingestion of low doses (up to 40 mg/kg bw) no signs of poisoning are on record. Mild poisoning (4.2-67 mg/kg bw) may result in nausea, vomiting and diarrhoea or shortlasting CNS depressions like dizziness, drowsiness and weakness. Severe poisoning after suicidal ingestion of high amounts of an abamectin formulation (equivalent to 38.5-227.3 mg/kg bw abamectin) resulted in a comatose state within 3 hours after ingestion, shock, respiratory failure and even death as a result of multiple organ failure. The dose of abamectin ingested orally by a patient with lethal outcome in suicidal intention was 88.1mg/kg. The maximum tolerated dose via the same route by another patient was 227.3mg/kg.

Clinical tests

No specific monitoring programs have been performed in humans.

98/8 Doc IIIA 6.12.6 section No.	Human case report (sensitization/allergenicity observation)	Official use only
91/414 Annex Point addressed	No corresponding Annex point	

Section A6.12.6 Annex Point IIA VI.6.9.6	Human case report (sensitisation/allergenicity observations)	
	No cases of poisoning have been reported to the company: No reports 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	

98/8 Doc IIIA 6.12.7 section No.	Human case report (specific treatment in case of accident or poisoning; first aid measures, antidotes and medical treatment, if known)	Official use only
91/414 Annex II Point addressed 5.9.5	Proposed treatment: first aid measures, antidotes, medical treatment	

Section A6.12.7 Annex Point IIA VI.6.9.7	Human case report (specific treatment in case of accident or poisoning; first aid measures, antidotes and medical treatment, if known)	Official use only

First aid measures and therapeutic regimes

Terminate exposure and 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. Contact a physician.
In case of ingestion:	Rapidly after exposure (<15 minutes) repeatedly administer medical charcoal in a large quantity of water or ipecac. If the patient is unconscious do not give anything by mouth and do not induce vomiting. Maintain and support respiratory function.

The therapy for abamectin poisoning is mainly symptomatic and supportive. Special attention should be given to maintain/support breathing.

Antidotes, Medical Treatment

Antidote	No antidote is known, apply symptomatic and supportive treatment
In case of skin/eye contact:	Decontamination
In case of ingestion:	Rapidly administer activated charcoal, ipecac and laxatives, gastric lavage. Maintain and support respiratory function.

Apply symptomatic and supportive therapy. Despite the lack of specific therapy, the follow-up of patients who had ingested a large amount of abamectin in suicide attempts showed an uneventful recovery. Therefore prognosis is good unless complications occur from severe uncontrolled aspiration.

98/8 Doc IIIA 6.12.8 section No.	**Human case report (prognosis following poisoning)**	**Official use only**
91/414 Annex **II** Point addressed **5.9.6**	Expected effects of poisoning Human case report	
Section A6.12.8 **Annex Point IIA VI.6.9.8**	Human case report (prognosis following poisoning)	**Official use only**

Expected effects of poisoning

There is a small database of 21 intoxicated persons whose symptoms are described below. Severe intoxications were observed after suicide attempts only.

Clinical data available from ingestion of abamectin formulations in suicide attempts show that, with doses up to 67 mg/kg, humans suffer from nausea, vomiting, diarrhoea, dizziness, drowsiness and weakness. All patients recovered within 2 days.

Higher doses may result in a comatose state within 3 hours after ingestion, transient hypotension, shock, complications of aspiration, respiratory failure and even death as a result of multiple organ failure. This group needs intensive symptomatic and supportive care but survivors will recover quickly and should be free of complications.

A treatment with ipecac or medical charcoal within 15 minutes of ingestion is able to prevent coma and death and other signs of abamectin intoxication in dogs, whereas a later administration of charcoal and ipecac is ineffective in reducing abamectin induced toxicity. It can be assumed that the same is valid for humans.

Commercially available formulations contain a low amount of the active ingredient only. Therefore severe intoxications are only possible if relatively large quantities of the formulation are ingested via the oral route or by deliberate injection with suicidal intent. Dermal exposure will lead to limited intoxication based on the low rate of absorption through the skin. The toxicity of co-ingested compounds (formulation agents, other active ingredients and alcohol) should also be taken into account.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*15 november 2007*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
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	

13. TOXICITY TO PETS/LIVESTOCK

Justification below not/partially reported in DAR

Section 6.13 Annex Point IIA 6.13	Toxic effects on livestock and pets	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [X]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*27March 2008*
Evaluation of applicant's justification	[redacted]

[redacted]

Conclusion	[redacted]
Remarks	

	COMMENTS FROM OTHER MEMBER STATE...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Adopt applicant's version or include revised version. If necessary, discuss relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Remarks	

14. OTHER TESTS RELATED TO HUMAN EXPOSURE

98/8 Doc IIIA 6.14 section No.	Other tests related to the exposure of humans

Section 6.14 Annex Point IIA 6.14	Other tests related to the exposure of humans	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [X]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*15 november 2007*
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Adopt applicant's version or include revised version. If necessary, discuss relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*

Remarks

15. FOOD AND FEEDING STUFFS

98/8 Doc IIIA 6.15 section No.	Food and feedingstuffs	Official use only

Section 6.15 Annex Point IIA 6.15	Food and feedingstuffs	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [X]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*27 March 2008*
Evaluation of applicant's justification	[redacted]

[redacted]

Conclusion	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Adopt applicant's version or include revised version. If necessary, discuss relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Remarks	

16. OTHER TESTS RELATED TO HUMAN EXPOSURE IN THE PRODUCT

98/8 Doc IIIA 6.16 section No.	Other tests related to the exposure of humans in the products	Official use only

Section 6.16 Annex Point IIA 6.16	Other tests related to the exposure of humans in the products	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [X]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*15 november 2007*
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Adopt applicant's version or include revised version. If necessary, discuss relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.*
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Remarks	

17. EFFECTS OF METABOLITES FROM TREATED PLANTS

98/8 Doc IIIA 6.17 section No.	Effects of metabolites from treated plants	Official use only

Section 6.17 Annex Point IIA 6.17	Effects of metabolites from treated plants	Official use only
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [X]	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*15 november 2007*
Evaluation of applicant's justification	[redacted]
Conclusion	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Adopt applicant's version or include revised version. If necessary, discuss relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and*

	conclusion.
Conclusion	Other conclusions: *(Adopt applicant's version or include revised version)*
Remarks	

Abamectin

Document IIIA

Section 7: Ecotoxicological Profile Including Environmental Fate and Behaviour

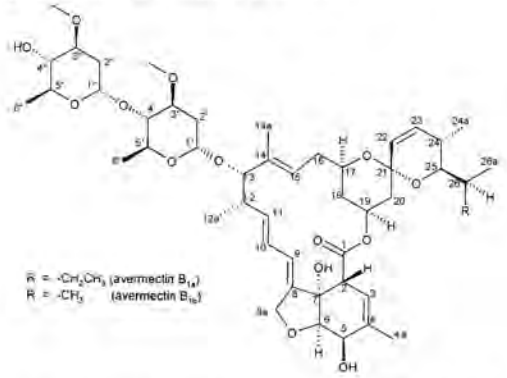
From
Tier I - Section 5 & 6 - Annex II
of 91/414 dossier:
Environmental /Ecotoxicity *studies* on the active substance

Introductory note:

Below is included the relevant parts of the 91/414/EEC Spinosad Draft Assessment Report written by the CA (NL-CTGB) and released in March 2008. All studies have been evaluated and accepted by the EU member states for Annex I inclusion under directive 91/414/EC. In the PPP area reliability indexes are not used, as there is no guidance to decide on the reliability. To fulfill BPD requirements the applicant has added text concerning GLP and reliability .

In the following summaries, the abbreviation for active substance ("as"), refers to abamectin, company code MK-0936. Abamectin, also known as avermectin B_1, is a mixture of two microbially produced compounds: avermectin B_{1a} (NOA 422601, ≥ 800 g/kg) and avermectin B_{1b} (NOA 421704, < 200 g/kg). The structural formula of abamectin is given in the following figure.

Figure: Structural formula of abamectin



In the following table, company codes for abamectin and related compounds are given. The names as used in the summaries are indicated in bold.

Table: Company codes for abamectin and related compounds.

Company code	Common name(s), composition
A-8612 A	**A-8612**, **Vertimec 018 EC**, **Vertimec 0.15 EC**: emulsifiable concentrate of abamectin, 18 g/L (15 lbs/gallon)
Dynamec	**abamectin 1.8 EC**
MK-0936, CGA 140327	**abamectin**, avermectin B_1
NOA 422601	**avermectin B_{1a}**
NOA 421704	**avermectin B_{1b}**
NOA 448111	**8a-oxo-avermectin B_{1a}**, 5-O-demethyl-8a-oxo-avermectin B_{1a}
NOA 448112	**8a-hydroxy-avermectin B_{1a}**, 5-O-demethyl-8a-hydroxy-avermectin B_{1a}
NOA 457464	**4,8a-dihydroxy-avermectin B_{1a}**, 4,8a-dihydroxy-$\Delta^{2,3}$-avermectin B_{1a},
NOA 457465	**8a-oxo-4-hydroxy-avermectin B_{1a}**, 8α- oxo-4-hydroxy-avermectin B_{1a}, 8α-oxo-4-hydroxy-$\Delta^{2,3}$-avermectin B_{1a},
NOA 427011	**[8,9-Z]-(isomer of)avermectin B_{1a}**, $\Delta^{8,9}$-isomer, delta 8,9-isomer, 8,9-cis-isomer
NOA 426289	**4"-oxo-avermectin B_{1a}**, 5-O-demethyl-4"-deoxy-4"-oxo-avermectin B_{1a}
NOA 445495	**3"-demethyl-avermectin B_{1a}**, 3",5-di-O-demethyl-avermectin B_{1a},
NOA 419150	**desoleandrosyl-avermectin B_{1a}**
DT1	**2-epi-avermectin B_{1a}**, 2-epi-NOA 422601
DT3	unnamed hydrolysis product
DT4	**1,18-hydrolysed avermectin B_{1a}**

98/8 Doc IIIA section No.	**7.1.1.1.1**	**Hydrolysis as a function of pH and identification of breakdown products**
91/414 Annex IIA point addressed	**7.2.1.1**	**Rate of hydrolysis**

		Official use only
Reference point in dossier	**7.1.1.1.1/01**	
Title:	Hydrolysis of [23-^{14}C]-NOA 422601 (Avermectin B_{1a})- under Laboratory Conditions	
Project/Report number:	99EH01	
Author(s):	Ellgehausen, H.	
Date of report:	08/02/2001	
Published:	Not published	
Testing facility:	Syngenta AG, Basel, Switzerland	
Study dates	20/9/1999 to 14/11/2000	
GLP:	Yes	
Reliability indicator	1	X

Reference/notifier	:	Ellgehausen, H. (2001)	GLP statement	:	yes
Type of study	:	hydrolysis	Guideline	:	OECD 111; US-EPA Subdivision N, 540/09-82-021, section 161-1 BBA 55, I and II
Year of execution	:	1999-2000	Acceptability	:	
Test substance	:	[23-^{14}C]-avermectin B_{1a}, batch [redacted], radiochemical purity [redacted]			

Substance	Buffer type	T [°C]	pH	Duration [d]	Transformation at end [%]	DT_{50} hydrolysis [d]	DT_{50} hydrolysis, 20 °C [d]
^{14}C-avermectin B_{1a}	phtalate	50	4	7	1.9	no hydrolysis	
	acetate	50	5	7	2.4	no hydrolysis	
	phosphate	50	7	7	2.8	no hydrolysis	
	borate	25	9	36	11.2	213	
	borate	50	9	25	82.6	9.9	380
	borate	60	9	11	77.4	4.9	

Description

Methods.

Pre-test. Standard solution is added to respective buffers, initial concentration 0.11 mg/L in 20 % acetonitrile. Six replicates incubated at 50 °C in the dark. Duplicate samples on days 0, 1, 2, 3, 4 and 7. To exclude oxidation, additional samples (0.12 mg/L) at pH 9 were incubated under N_2 at 60 °C, sampling on day 1 and 4. Temperature, pH and sterility confirmed.

Final test. Incubation of 0.11 mg/L at pH 9, 25, 50 and 60 °C for 36, 25 and 11 days, respectively. Temperature, pH and sterility confirmed. For isolation of degradation products, a 400 mL solution of 0.123 mg/L at pH 9 was incubated at 60 °C for three days.

Chemical analysis. Samples analysed by LSC, and extracted three times with ethylacetate. Combined organic phases concentrated, residues dissolved in acetonitrile/water and analysed by HPLC-UV (244 nm) and 2D-TLC.

Isolation of degradation products achieved by four times extraction with ethylacetate, combined extracts concentrated, taken up in acetonitrile/water, fractionated by HPLC or TLC. Final purification by repeated TLC, identification by LC-NMR and LC-MS.
Calculations. $DT_{50,hydrolysis}$ estimated by non-linear fit of first order kinetics, formation and decline of first transient degradation product with ModelMaker 3.03. Arrhenius constants derived by linear regression of ln(k) versus 1/T, Arrhenius equation used to calculated $DT_{50,hydrolysis}$ at 20 °C.

Results

Pre-test. Recovery of radioactivity 98.4 - 99.8 % of AR, 1.9 - 2.8 % degradation at pH 4 - 7, 36.3 % at pH 9.
Final test. Recovery 99.3 - 99.8 % of AR. $DT_{50,hydrolysis}$ 212.6, 9.9 and 4.9 days at 25, 50 and 60 °C. $DT_{50,hydrolysis}$ at 20 °C determined as 379.9 days, based on Arrhenius constants (Ea 21.7 kcal/mole, A 2.9 x 10^{13}/s). Major degradation product DT1 was identified as 2-Epi-avermectin B_{1a}, with maximum amounts of 24.6 and 25.4 % of AR at 50 and 60 °C. DT_{50} for this compound was estimated as 4.4 days at 50 °C and 1.5 days at 60 °C. DT3 and DT4 (1,18-hydrolysed avermectin B_{1a}) represent stable end-products with levels of 15.6 and 17.5 % of AR at 60 °C.

Remarks by RMS

According to text, M1/DT1 was detected at 50 °C. In the table with HPLC results, maximum of M1/DT1 is given as 63.8 % of AR after 25 days. In the tables with TLC results, M1/DT1 is not mentioned and its reported maximum of 24.6 % of AR is given for M2/DT2. Codes have probably been mixed up. Reported maximum levels for DT1 refer to TLC-results, HPLC values are higher: 63.8 % of AR after 9 days at 50 °C, and 57.9 % of AR after 9 days at 60 °C. Amounts of avermectin B_{1a} as determined by HPLC and TLC are similar. The results no hydrolysis of avermectin B_{1a} at pH 4, 5 and 7, and $DT_{50,hydrolysis}$ 380 days at pH 9, 20 °C (calculated value), and information on metabolites are used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks

		Official use only
Reference point in dossier	**7.1.1.1.1/02**	
Title:	Hydrolysis of Avermectin B_{1a} (MK-0936)	
Project/Report number:	MSM12087	
Author(s):	Maynard, M. S. and Ku, C. C.	
Date of report:	15/09/1982	
Published:	Not published	
Testing facility:	Merck Sharp and Dohme; Rahway, N.J., USA	
Study dates	14/5/1982 to 11 June 1982	
GLP:	No	
Reliability indicator	1	

Reference/notifier	:	Maynard, S. and Ku, C.C. (1982)	GLP statement	:	no
Type of study	:	hydrolysis	Guideline	:	US-EPA Subdivision N, 161-1, proposal 1982
Year of execution	:	1982	Acceptability	:	acceptable
Test substance	:	[5-^{3}H]-avermectin B_{1a}, radiochemical purity [redacted]			

Substance	Buffer type	T [°C]	pH	Duration [d]	Transformation at end [%]	DT_{50} hydrolysis [d]	DT_{50} hydrolysis, 20 °C [d]
^{3}H-avermectin B_{1a}	phopshate	25	5	28	0	-	-
	phosphate	25	7	28	0	-	-
	borate	25	9	28	4.8	-	-

Description

Methods.
Test solutions of 10 mg/L were prepared in buffers with 2 % formulation solution (88 % propylene glycol and 12 % witconol 1206). Incubation for 28 days at 25 ± 1 °C in the dark. Single samples were taken after 0, 4, 7, 13 and 28 days and analysed by LSC and HPLC-UV (245 nm), pH was checked.

Results

Actual pH 5.6, 6.8 and 8.6. Recovery 93.4 - 111 % of day 0 values. No decline of avermectin B_{1a} concentrations with time.

Remarks by RMS

The result no hydrolysis of avermectin B_{1a} at pH 5, 7 and 9, 25 °C is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*

Materials and Methods Results and discussion Conclusion Reliability Acceptability Remarks	[redacted]
	COMMENTS FROM ...
Date Materials and Methods Results and discussion Conclusion Reliability Acceptability Remarks	

98/8 Doc IIIA section No.	7.1.1.1.2	Photo-transformation in water including identity of products of transformation

		Official use only
Reference point in dossier	**7.1.1.1.2/01**	
Title:	Aqueous Photolysis of [23-^{14}C] - labelled NOA 422601 (Avermectin B_{1a}) under Laboratory Conditions	
Project/Report number:	01DA01	
Author(s):	Adam D.	
Date of report:	14/11/2001	
Published:	Not published	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	09/01/2001 to 29/06/2001	
GLP:	Yes	
Reliability indicator	1	

Reference/notifier	:	Adam, D. (2001b)	GLP statement	:	yes
Type of study	:	photodegradation in water	Guideline	:	US-EPA Subdivision N, 161 - 2 EPA 540/09-90-078
Year of execution	:	2001	Acceptability	:	acceptable
Test substance	:	[23-^{14}C]-avermectin B_{1a}, batch [redacted] radiochemical purity [redacted]			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [d]	Quantum yield	Transformation at end [%]	DT_{50} photo [d]
^{14}C-avermectin B_{1a}	phosphate buffer	25	7	Xenon	> 290	37.5		90	2

Description

Methods. Test solutions were prepared in phosphate buffer with 1% acetonitrile, final concentration ca. 0.1 mg/L. Samples were irradiated in a Suntest exposure unit with a Xenon lamp with UV-filter (λ > 290 nm), 12:12 hours L:D. Incubation for 37.5 days. Duplicate samples were taken at regular time points and diluted to 20 % acetonitrile. Volatiles were trapped in ethylene glycol and 2 N NaOH. Light intensity was monitored, sterility of buffers confirmed.

Chemical analysis. Solutions were analysed by LSC, 2D-TLC and/or HPLC-UV (245 nm). Trapping solutions were analysed by LSC, CO_2 confirmed by $BaCO_3$-precipitation. Reference compounds: avermectin B_{1a}, NOA 427011 (8,9-Z avermectin B_{1a}), NOA 488111 and NOA 488112.

Calculations. $DT_{50,photolysis}$ of avermectin B_{1a} estimated by non-linear regression of first order kinetics, rate constants for formation and decline of metabolite NOA 427011 estimated by non-linear fit of series first-order kinetics.

Results

Light intensity was on average 38.8 W/m^2, corresponding value of summer midday sunlight at 30 – 50 °N is 67 ± 0.3 w/m^2, ratio of intensity 0.58. Irradiation (12 h) corresponded to 0.77 days at 30 - 50 °N. Recovery in dark controls 96.6 - 100.7 % of AR, avermectin B_{1a} accounted for 91.9 % of AR and NOA 448111 for 2.4 % of AR after 37.5 days. Distribution of radioactivity in irradiated samples is given in the Table below.

Table: Distribution of radioactivity in irradiated samples. All values are % of AR.

Time [d]	Solution	CO_2	Total	Avermectin B_{1a}	NOA 448111	NOA 427011
0	99.2	-	99.2	91.8	2.1	< LOD
1 h	98.8	< LOD	98.8	89.9	1.6	2.3
3 h	97.4	< LOD	97.4	87.2	2.6	4.0
7 h	98.2	< LOD	98.2	75.2	4.1	5.8
13 h	99.3	< LOD	99.4	72.6	4.3	**8.2**
2	99.6	< LOD	99.6	55.8	5.2	7.4
4	97.4	1.5	97.5	11.7	4.5	6.8
6	97.3	0.1	97.5	9.3	3.9	4.6
12	95.6	0.5	96.1	4.1	2.7	n.a.
18	95.5	1.0	96.5	2.3	**5.6**	n.a.
24	92.6	1.1	93.7	1.9	5.1	n.a.
37.5	89.8	2.4	92.3	1.6	2.9	n.a

n.a. = not analysed

Up to 30 unknown fractions were detected, individual compounds accounted for at most 6.6 % of AR.
$DT_{50,photolysis}$ for avermectin B_{1a} was determined as 24 irradiated hours, equivalent to 1.5 midsummer sunlight days at 30 - 50 °N. $DT_{50,photolysis}$ for NOA 427011 estimated as 41.1 hours, equivalent to 2.7 days at 30 - 50 °N.

Remarks by RMS

Recalculation of $DT_{50,photolysis}$ for avermectin B_{1a} gives same result, $DT_{50,photolysis}$ for NOA 427011 is estimated as 7.6 days taking maximum level as starting point (n = 4; equivalent to 5.8 sunlight days at 30 - 50 °N). The following results are used for risk assessment:

- $DT_{50,photolysis}$ for avermectin B_{1a} 2 days (1.5 sunlight days at 30 - 50 °N)
- 5.6 % formation of NOA 448111 and 8.2 % formation of [8,9-Z]-avermectin B_{1a}
- $DT_{50,photolysis}$ for [8,9-Z]-avermectin B_{1a} 7.6 days (5.8 sunlight days at 30 - 50 °N).

Syngenta endpoint(s) in originally submitted Document III A Section 7:
$DT_{50,photolysis}$ for [8,9-Z]-avermectin B_{1a} / NOA 427011 estimated as 41.4 hours, equivalent to 2.7 days at 30 - 50 °N.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
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Date	

Materials and Methods
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

		Official use only
Reference point in dossier	**7.1.1.1.2/02**	
Title:	Photodegradation of Avermectin B_{1a} in water and soil environment	
Project/Report number:	unknown	
Author(s):	Ku, C.C, Jacob, T.A.	
Date of report:	31/10/1983	
Published:	Not published	
Testing facility:	Merck & Co. Inc., Rahway NJ, United States	
Study dates	1983	
GLP:	No	
Reliability indicator		

Reference/notifier	:	Ku, C.C. and Jacob, T.A. (1983b)	GLP statement	:	no
Type of study	:	photodegradation in water	Guideline	:	US-EPA Subdivision N, 161 - 2
Year of execution	:	1983	Acceptability	:	DT_{50}: not acceptable identity of metabolites: acceptable
Test substance	:	[5-^{3}H]-avermectin B_{1a}, radiochemical purity > [redacted] [3,7,11,13,23-^{14}C]-avermectin B_{1a}, radiochemical purity [redacted]			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [h]	Quantum yield	Transformation at end [%]	DT_{50} photo [d]
^{14}C-avermectin B_{1a}	distilled water			natural sunlight		29		97.4 - 100	

Description

Photodegradation under natural sunlight. A solution of 700 mg/L ^{14}C-avermectin B_{1a} (labelled at C3, 7, 11, 13 and 23) was prepared in acetonitrile, 0.5 mL of this solution was dispersed in 49.5 mL distilled water to give a concentration of ca. 7 mg/L in 1 % acetonitrile. Two flasks were exposed to sunlight at Three Bridges, New Jersey, USA, 40.5 °N in August 9 to 15, 1983. One aluminium wrapped flask was kept as dark control. Sunlight intensity and temperature were monitored. Water samples were taken at regular time intervals during 29 hours, taken up in methanol and analysed by reversed phase HPLC-UV (254 nm) and LSC. Identification of metabolites by MS and NMR.

Comparison of ^{3}H and ^{14}C. A solution of 20 mg/L avermectin B_{1a} in 48 % acetonitrile and 50 % water was exposed to a sunlamp, 2 % acetone was added as a photosensitiser.

Preparation of photodegradation products. For purposed of identification, and equimolar mixture of $^{1}H/^{2}H$-labelled avermectin B_{1a} with traces of ^{3}H-avermectin B_{1a} was dispersed at a concentration of 240 mg/L in acetone/acetonitrile/water (2/58/40, v/v/v). The solution was placed under a sunlamp, photodegradation products

were isolated and purified by reversed phase HPLC. Identification of metabolites by MS and proton nuclear resonance (^{1}H-NMR).

Results

Photodegradation under natural sunlight. Remaining ^{14}C-avermectin B_{1a} in dark control and irradiated flasks over time is given in the table below. Half-lives were calculated as 12 and 3.5 h for replicate 1 and 2, respectively.

Table: Remaining ^{14}C-avermectin B_{1a} in dark control and irradiated samples.

Exposure time [h]	Dark control	Replicate 1	Replicate 2
0	100	100	100
1	101	95.7	79.1
2	95.4	90.1	67.1
4	103	81.9	43.9
8	83.3	61.8	17.3
12	92.2	51.7	5.6
15	94.2	40.9	0
20	79.1	24.8	0
29	75.6	2.6	0

Comparison of ^{3}H and ^{14}C. Ratios between polar, moderately polar and non-polar degradation products were similar for ^{3}H- and ^{14}C-labelled avermectin B_{1a}. Maximum levels of polar, moderately polar and non-polar peaks are 55.2 % after 78 hours, 38.5 % after 30 hours and 12.0 % after 4 hours, respectively. NMR and MS analyses indicated that the non-polar fractions consisted mainly of a single component, whereas multiple components were present in the polar and moderately polar fractions. It was concluded that the polar fraction retained both the ^{3}H and ^{14}C-atoms. HPLC-pattern was similar to that of the first experiment, indicating that presence of sensitiser acetone did not change photodegradation process.
Identification of metabolites. Mass spectrum of non-polar peak was similar to that of avermectin B_{1a}: ions are found at m/z 113, 145 and 257, arising from sugar portion of the molecule, at m/z 193 and 305, arising from the upper portion of the macrocyclic system, and m/z 566 and 567, ion doublet, which is the aglycone minus water, the doublet is from ^{1}H/^{2}H-equimolar mixture at C5. It is concluded that the non-polar product is isomeric with avermectin B_{1a}, which was confirmed by ^{1}H-NMR analyses. From several possibilities, the Δ^{8-9} isomeric structure (= [8,9-Z]-isomer) was found to explain the differences in the proton spectrum. The moderate polar fraction could not be identified, the polar fraction contained multiple components with an intact sugar moiety.
In an addendum it is stated that the non-polar and moderately polar fractions are transient metabolites that are further degraded into the polar fraction. This fraction contains multiple peaks and, according to an internal memo, is > 160 times less toxic to *Daphnia magna* than avermectin B_{1a}.

Remarks by RMS

Temperature and light intensity not given. Difference between replicates in first experiment indicates that light conditions were not comparable. DT_{50}'s are not used for risk assessment. Description of identification results is based on text, figures are very unclear. From one of the (unclear) figures, the polar fraction seems to consist of three peaks. Fraction increases to 55.2 % at the end of the experiment, and maximum formation is likely not reached by then. It is thus assumed that individual compound are likely to be formed in substantial amounts. The moderately polar fraction contains one peak. The results 12 % formation of [8,9-Z]-avermectin B_{1a} and > 10 % formation of unknown metabolites is used for risk assessment.

Syngenta endpoint(s) in originally submitted Document III A Section 7:
The polar and moderately polar fractions contained multiple components.

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*

Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

		Official use only
Reference point in dossier	**7.1.1.1.2/03**	
Title:	The photodegradation of [^{3}H]avermectin B_{1a} under sunlight	
Project/Report number:	AEDM-732	
Author(s):	Halley, B.A., Andrew, N., Green-Erwin, M., Narasimhan, N.I.	
Date of report:	02-05-1991	
Published:	Not published	
Testing facility:	Merck & Co, Inc.	
Study dates		
GLP:	Yes	
Reliability indicator	1	

Reference/notifier	:	Halley, B.A., Andrew, N., Green-Erwin, N. and Narasimham, N.M. (1991)	GLP statement	:	yes
Type of study	:	photodegradation in water	Guideline	:	US-EPA Vol. 50, 188 (1985)
Year of execution	:	1990	Acceptability	:	acceptable
Test substance	:	[5-^{3}H]-avermectin B_{1a}, batch [redacted] radiochemical purity [redacted]			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [d]	Quantum yield	Transformation at end [%]	DT_{50} photo [d]

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [d]	Quantum yield	Transformation at end [%]	DT_{50} photo [d]
^{3}H-avermectin B_{1a}	sterile water	19		natural sunlight	> 290	6	0.0287 – 0.0347	72	1.3

Description

Methods. Stock solutions in methanol were diluted in pure sterile 18 megohm water with 1% acetonitrile, final concentration ca. 3 µg/L. Samples were incubated for 6 days under natural daylight conditions. Dark controls were wrapped in foil. Triplicate samples were taken at regular time points. 4'-Nitroacetophenone (PNAP; 1.656 mg/L) and pyridine (PYR; 7 mL/L) were used as positive controls as recommended by the EPA. Weather conditions, and temperature in the samples and surrounding air were recorded.
Chemical analysis. Unlabelled abamectin (lot L-676,863-00V084, 97 % pure) was added as carrier. Samples evaporated to dryness, resuspended in methanol:water (90:10) and analysed by HPLC-UV (245 nm).

Results

Minimum air temperature 6.1 – 12.8 °C, maximum temperature 27.2 – 32.8 °C, overall average 18.7 °C. Temperature in the samples, measured around noon each day, was between 22.0 and 32.0 °C, average 26.6 °C. Samples received appr. eight hours of sunlight/day.
Average recovery of radioactivity (± SD) before HPLC was 112.6 ± 10.9 % of AR, after HPLC average recovery was 87.7 ± 15.2 % of AR. Average recovery of avermectin B_{1a} is given in Table B.8.4.2-3.

Table B.8.4.2-3. Recovery of avermectin B_{1a} in irradiated samples and dark controls.

Time [d]	Irradiated	Dark
0	93.3	99.3
0.25	80.5	-
0.5	71.9	-
1	39.6	89.9
2	23.6	96.4
3	18.7	94.2
4	19.4	97.8
5	14.7	97.9
6	21.6	90.9

Authors conclude that degradation of avermectin B_{1a} follows biphasic kinetics, the fast phase between day 0 and 1 is partly attributed to isomerisation and a slower phase as from day 1 is arising from photodegradation to polar products. DT_{50} was estimated as 0.32 and 3.16 days for the first and second phase, respectively, applying non-linear fit of two-phasic first order kinetics. $DT_{50,photolysis}$ was estimated as 3.2 days applying linear regression on ln-transformed day 1 to 5 versus time. From the ratio between the rate constants of avermectin B_{1a} and PNAP, the absorbance data, solar intensities (40 °N) and the quantum yield of PNAP, the quantum yield of avermectin B_{1a} photodegradation was determined to be 0.0347 (summer), 0.0316 (fall) and 0.0287 (winter). Corresponding $DT_{50,photolysis}$ values for summer, fall and winter at a flat water surface under clear skies were 1.32 days, 2.88 days and 5.08 days, respectively.

Remarks by RMS

Assumption of first isomerisation-phase is based on other studies. No identification of metabolites. Recalculation of $DT_{50,photolysis}$ for avermectin B_{1a} with non-linear fit of first order kinetics and using all data, gives value of 1.3 days with acceptable regression coefficient (r^2 0.9048). This value is used for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	EVALUATION BY RAPPORTEUR MEMBER STATE *27-06-2007*
Materials and Methods	
Results and discussion	
Conclusion	[redacted]

Reliability Acceptability Remarks	[redacted]
	COMMENTS FROM ...
Date Materials and Methods Results and discussion Conclusion Reliability Acceptability Remarks	

98/8 Doc IIIA section No.	**7.1.1.2.1**	**Ready biodegradability**
91/414 Annex IIA point addressed	**7.2.1.3.1**	**Ready biodegradability**

		Official use only
Reference point (location) in dossier	**7.1.1.2.1/01**	
Title:	Ready Biodegradability of MK 936 (Abamectin) - (Manometric Respirometry Test)	
Project/Report number:	G 557 06	
Author(s):	Dietschy, A.	
Date of report:	05/02/1999	
Published:	Not published	
Testing facility:	Novartis Services AG, Ecotox Center, Basel, Switzerland	
Study dates	21/10/1998 to 22/12/1998	
GLP:	Yes	
Reliability indicator	1	

Reference/notifier	:	Dietschy, A. (1999)	GLP statement	:	yes
Type of study	:	ready biodegradability	Guideline	:	OECD 301 F
Year of execution	:	1998	Acceptability	:	acceptable
Test substance	:	abamectin, batch [redacted] chemical purity [redacted] white powder			

Substance	Water type	T [°C]	pH	Duration [d]	Transformation at end [%]	Classification
abamectin	activated sludge	22 – 23	7.3 – 8.2	28	3	not readily biodegradable

Description

Manometric respiromety test.
Methods. Test substance was added to a mineral salts medium inoculated with activated sludge, suspended solids concentration 26 mg/L, test concentration 100 mg/L. Duplicate vessels for test substance (medium + abamectin), blank control (medium + inoculum), positive control (medium + sodium benzoate), abiotic control (medium without inoculum + abamectin + sterilising agent $Hg(II)Cl_2$ and toxicity control (medium + abamectin + sodium benzoate). Incubation for 28 days in the dark. BOD was measured continuously.
Calculations. Biodegradation calculated as BOD/Theoretical Oxygen Demand.

Results

Oxygen consumption in blank control 14 – 20 mg/L after 28 days. Biodegradation of abamectin was 3 % after 28 days. Biodegradation of sodium benzoate was 89 % at day 14 and 95 % after 28 days, biodegradation in toxicity control was 77 and 81 % after 14 and 28 days. Abiotic control had 1 % degradation after 28 days.

Remarks by RMS

Validity criteria are met. The result that abamectin is *not readily biodegradable* is used for risk assessment.

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

98/8 Doc IIIA section No.	7.1.1.2.2	Inherent biodegradability, where appropriate

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*24-10-2007*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
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98/8 Doc IIIA section No.	7.1.1.2.3	Biodegradation in seawater

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [X]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	EVALUATION BY RAPPORTEUR MEMBER STATE *24-10-2007* [redacted]	
Date Evaluation of applicant's justification Conclusion Remarks	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

98/8 Doc IIIA section No.	7.1.2.1.1	Aerobic biodegradation

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*24-10-2007*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
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98/8 Doc IIIA section No.	7.1.2.1.2	Anaerobic biodegradation

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*24-10-2007*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
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Conclusion		
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98/8 Doc IIIA section No.	7.1.2.2.1	Aerobic aquatic degradation study

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] Limited exposure [ ]	Technically not feasible [ ] Scientifically unjustified [ ] Other justification [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
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Date	*24-10-2007*	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

98/8 Doc IIIA section No.	**7.1.2.2.2**	**Water/sediment degradation study**
91/414 Annex IIA point addressed	**7.2.1.3.2**	**Water/sediment study**

		Official use only
Reference point (location) in dossier	**7.1.2.2.2/01**	
Title:	Metabolism and rate of degradation of [23-^{14}C]-labelled NOA 422601 (Avermectin B_{1a}) under aerobic and anaerobic laboratory conditions in aquatic systems	
Project/Report number:	01TB01	
Author(s):	Buckel, T.	
Date of report:	26/07/2002	
Published:	Not published	
Testing facility:	Syngenta Crop Protection AG, Basel, Switzerland	
Study dates	8/6/2001 to 12/4/2002	
GLP:	Yes	
Reliability indicator	1	

Reference/notifier	:	Buckel, T. (2002)	GLP statement	:	yes
Type of study	:	water/sediment degradation	Guideline	:	OECD draft 2000; BBA IV, 5-1
Year of execution	:	2001-2002	Acceptability	:	acceptable
Test substance	:	[23-^{14}C]-avermectin B_{1a}, batch [redacted] radiochemical purity [redacted]			

Substance	Sediment type	Conditions	Ratio sediment water [g dwt/mL]	T [°C]	pH	OM [%]	Duration [d]	Degradation at end [%]	DT_{50} water [d]	DT_{50} sediment [d]	DT_{50} system [d]
^{14}C-avermectin B_{1a}	sandy loam	aerobic	0.20	20	7.9-8.4	2.5	100	53	1.8[1]	87	87
^{14}C-avermectin B_{1a}	sandy loam	anaerobic	0.20	20	8.4-9.2	2.6	100	25	7.2[1]		230[2]
^{14}C-avermectin B_{1a}	silty clay loam	aerobic	0.12	20	7.7-8.4	7.7	100	53	2.9[1]	111	91
^{14}C-avermectin B_{1a}	silty clay loam	anaerobic	0.12	20	7.8-9.8	7.8	100	18	5.6[1]		312[2]

1: $DT_{50,water}$ determined by sorption, value represents dissipation
2: extrapolated value

Description

Water/sediment systems. Two types of aerobic and anaerobic water sediment systems were set up: Sandy loam. River Rhine (Möhlin, CH): 120 g dwt sediment, corresponding to 228 g wwt for aerobic and 219 g wwt for anaerobic (16 % sediment). CEC 105 mmol/kg, 1.48 – 1.52 % OC, pH-$CaCl_2$ 7.2 for sediment, 8.1 for water. Microbial biomass aerobic sediment 341 mg C/kg at start of test, anaerobic plate counts 2.8 x 10^5/g dwt. Silty clay loam. Natural pond (Rothenfluh 3.2, CH): 80 g dwt sediment, corresponding to 237 g wwt for aerobic and 239 g wwt for anaerobic (11 % sediment). CEC 308 - 315 mmol/kg, 4.52 – 4.58 % OC, pH-$CaCl_2$ 7.1 for sediment, 8.0 for water. Microbial biomass aerobic sediment 1440 mg C/kg at start of test, anaerobic plate counts 2.3 x 10^6/g dwt.
Sediment samples taken from 3 – 10 cm depth (aerobic) and 20 – 30 cm (anaerobic), wet sieved (2 mm). Incubation flasks (Ø 10 cm) filled with 2 – 2.5 cm wet sediment and ca. 500 mL (6 – 6.5 cm) corresponding

water. All handling for anaerobic part carried out under exclusion of oxygen. Equilibration for 28 days at 20 ± 2 °C in the dark under ventilation with air (aerobic) or N_2 (anaerobic).

Application, incubation and sampling. Test substance was applied as a solution in acetonitrile (240 µL) into the water phase, total amount 0.045 mg per system (0.09 mg/L overlying water). Three additional aerobic systems were applied with 0.14 mg per system for isolation of metabolites. Incubation at 20 ± 2 °C in the dark, pH, redox potential and oxygen content were monitored. Water and sediment were sampled in duplicate after 0, 3, 7, 14, 35, 70 and 100 days. Volatiles were trapped in ethylene glycol and 2N NaOH. Microbial biomass of aerobic sediment determined according to Anderson and Domsch at start and end, for anaerobic systems CFU's determined by anaerobic plate count method.

Chemical analysis.

Water. Radioactivity determined by LSC after 1:1 dilution with acetonitrile. Analysis by 2D-TLC and/or HPLC-UV (243 nm) after concentration (recovery 90 – 110 %).

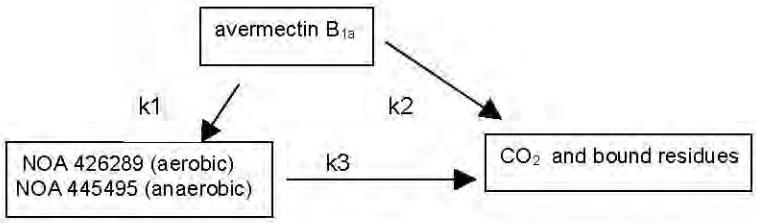
Sediment. Extraction three times by shaking with acetonitrile (200 rpm) for 30 min at room temperature. Radioactivity in extract determined after centrifugation (2000 rpm, 10 min, 10 °C), extracts combined. Sediment further extracted with acetonitrile under reflux for two hours, extracts counted by LSC and added to the cold extracts. Combined extracts analysed by LSC, 2D-TLC and/or HPLC after concentration (recovery 90 – 110 %). Additional harsh extraction for day-100 aerobic samples by reflux with acetonitrile/water (4:1), followed by reflux with acetonitrile/0.1 N HCl, both at 80 °C for two hours. Bound residues analysed by LSC after combustion. Sediment organic matter fractionation by precipitation with NaOH and HCl.

Volatiles. Radioactivity in trapping solutions determined directly by LSC, CO_2 confirmed by $BaCO_3$-precipitation.

Reference compounds: avermectin B_{1a}, NOA 448111, NOA 448112, desoleandrosyl-avermectin B_{1a} (NOA 419150), 2-epi-avermectin B_{1a}, 4"-oxo-avermectin B_{1a} (NOA 426289), (3"-demethyl-avermectin B_{1a} (NOA 445495), hydroxymilbemycin, and milbemycin D. LOQ (selected samples, defined as 3 x background): 0.3 % of AR (LSC), 0.4 – 3 µg/kg (TLC), 1.1 – 2.5 µg/kg (HPLC). Identification of metabolites by LC-NMR and LC-MS.

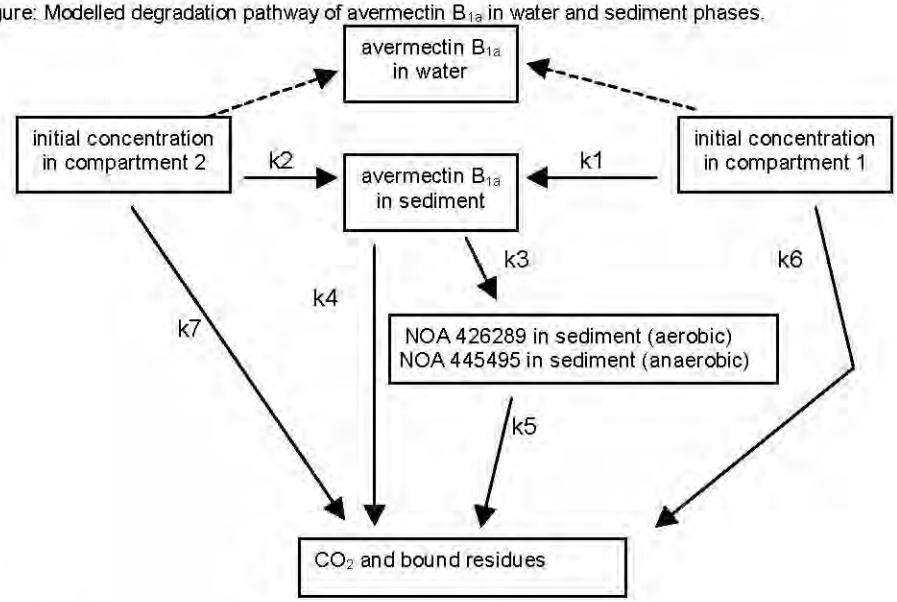
Calculations. Degradation of avermectin B_{1a}, simultaneous formation and decline of metabolites and formation of CO_2 and bound residues was modelled in ModelMaker 3.0, half-lives were determined by non-linear fit of first order kinetics. The following reaction scheme was used for the total system:

Figure: Modelled degradation pathway of avermectin B_{1a} in water/sediment systems.



The DT_{50} of avermectin B_{1a} in the water-phase and formation and decline of metabolites in sediment was estimated assuming a two-compartment model as presented in the following scheme:

Figure: Modelled degradation pathway of avermectin B_{1a} in water and sediment phases.



Results

Sandy loam (River).

Aerobic incubation. Microbial biomass at end 37.5 mg C/100. Redox potential 10 - 110 mV (water) and -151 - -494 mV (sediment). DO 4.2 - 6.4 mg/L, pH water 7.9 - 8.4.

Anaerobic incubation. Plate counts at end 2.48 x 10^5 CFU/g. Redox potential -101 - 400 mV (water) and -470 - -606 mV (sediment), DO max. 0.1 mg/L, pH water 8.4 - 9.2.

Distribution of radioactivity for the aerobic incubation is given in the following table for parent and metabolites > 5 % of AR. Organic volatiles were < LOD.

Table: Distribution of radioactivity in sandy loam system (River) after **aerobic** incubation. All values represent % of AR.

Compartment/fraction	Incubation period [d]						
	0	3	7	14	35	70	100
Water							
total radioactivity	93.0	22.5	18.5	11.1	6.7	8.4	10.2
Sediment							
Extractable[1]	7.1	74.7	80.5	85.2	**85.8**	67.5	60.8
Non-extractable	0.1	1.4	2.2	3.1	6.4	15.7	**20.4**
CO_2	< LOD	0.0	0.0	0.1	0.4	1.7	**3.0**
Total	100.2	98.7	101.4	99.5	99.3	93.3	94.3
avermectin B_{1a}							
water	92.0	21.7	17.4	9.8	4.6	1.5	1.9
sediment	7.0	73.4	79.0	**82.8**	78.4	50.2	44.3
total system	99.0	95.0	96.4	92.6	83.0	51.7	46.2
NOA 426289							
water	0.2	0.2	0.1	0.1	0.1	0.2	**0.3**
sediment	ND	0.3	0.2	0.3	1.7	**6.9**	5.4
total system	0.2	0.5	0.2	0.4	1.8	**7.0**	5.7

1: cold and reflux combined
ND: below limit of detection

Metabolites NOA 448111, NOA 448112 and NOA 445495 were found in low levels: < 1 % of AR in water phase and max. 1.9 – 2.1 % of AR in sediment. The remaining fraction (maximum for whole system 9 % of AR) consisted of at least 14 individual compounds. Distribution of radioactivity for the anaerobic incubation is given in the following table for parent and metabolites > 5 % of AR. Organic volatiles were < LOD.

Table: Distribution of radioactivity in sandy loam system (River) after **anaerobic** incubation. All values represent % of AR.

Compartment/fraction	Incubation period [d]						
	0	3	7	14	35	70	100
Water							
total radioactivity	92.2	45.4	39.7	29.2	20.6	13.4	12.1
Sediment							
Extractable[1]	6.8	51.8	58.8	68.3	75.5	77.1	**80.7**
Non-extractable	0.1	0.9	1.1	1.5	2.7	3.3	**4.9**
CO_2	< LOD	0.0	0.0	0.0	0.0	**0.1**	**0.1**
Total	99.1	98.0	99.7	99.1	98.8	93.9	97.8
avermectin B_{1a}							
water	92.0	44.8	38.8	27.9	18.5	9.9	8.6
sediment	6.7	51.0	57.7	66.2	**70.8**	66.7	65.5
total system	98.7	95.9	96.5	94.1	89.4	76.6	74.1
NOA 445495							
water	ND	ND	0.3	0.3	0.7	1.2	**1.6**
sediment	ND	0.1	0.3	0.9	2.8	7.7	**10.2**
total system	ND	0.1	0.5	1.2	3.5	8.8	**11.8**

1: cold and reflux combined
ND: below limit of detection

Metabolites NOA 448111, NOA 448112 and NOA 426289 were < 1 % of AR in water and sediment phase.

Silty clay loam (Pond).

Aerobic incubation. Microbial biomass at end 135 mg C/100. Redox potential 15 - 120 mV (water) and -235 - -480 mV (sediment). DO 4.3 - 7.2 mg/L, pH water 7.7 - 8.4.

Anaerobic incubation. Plate counts at end 2.48 x 10^5 CFU/g. Redox potential -54 - -391 mV (water) and -425 - -560 mV (sediment), DO max. 0.1 mg/L, pH water 7.8 - 9.8.

Distribution of radioactivity for the aerobic incubation is given in the following table for parent and metabolites > 5 % of AR. Organic volatiles were < LOD.

Table: Distribution of radioactivity in silty clay loam system (Pond) after **aerobic** incubation. All values represent % of AR.

Compartment/fraction	Incubation period [d]						
	0	3	7	14	35	70	100
Water							
total radioactivity	85.3	27.7	17.1	17.1	8.0	3.9	5.5
Sediment							
Extractable[1]	14.3	70.9	77.4	80.7	**81.8**	70.4	64.5
Non-extractable	0.2	1.4	2.3	3.1	7.8	16.4	**23.2**
CO_2	< LOD	0.0	0.0	0.1	0.4	1.6	**3.2**
Total	99.8	100.0	96.8	101.0	98.1	92.4	96.5
avermectin B_{1a}							
water	85.0	27.1	15.5	15.0	4.5	1.2	1.0
sediment	14.1	70.5	75.6	**78.1**	71.9	56.8	45.3
total system	99.1	97.6	91.1	93.1	76.4	57.9	46.3
NOA 426289							
water	ND	0.1	0.2	0.1	**0.3**	0.2	**0.3**
sediment	ND	0.1	0.5	0.8	6.1	6.5	**8.6**
total system	ND	0.1	0.7	1.0	6.4	6.6	**8.9**

1: cold and reflux combined
ND: below limit of detection

Metabolites NOA 448111, NOA 448112 and NOA 445495 were < 1 % of AR in the water phase and max. 1.5 – 2.8 % of AR in sediment. The remaining fraction (maximum for whole system 5.4 % of AR) consisted of at least 14 individual compounds. Distribution of radioactivity for the anaerobic incubation is given in the following table. Organic volatiles were < LOD.

Table: Distribution of radioactivity in silty clay loam system (Pond) after **anaerobic** incubation. All values represent % of AR.

Compartment/fraction	Incubation period [d]						
	0	3	7	14	35	70	100
Water							
total radioactivity	89.6	43.7	33.3	26.0	15.9	6.4	4.9
Sediment							
Extractable[1]	10.5	54.8	65.3	73.4	83.1	82.9	**88.7**
Non-extractable	0.2	0.9	1.2	1.6	2.1	**5.1**	4.3
CO_2	< LOD	< LOD	0.0	0.0	0.0	**0.1**	**0.1**
Total	100.3	99.4	99.9	101.1	101.1	94.4	98.0
avermectin B_{1a}							
water	89.6	43.2	32.6	25.0	14.0	5.2	3.6
sediment	10.4	54.2	64.2	71.6	**79.3**	75.3	78.2
total system	100.0	97.4	96.8	96.6	93.3	80.5	81.7
NOA 445495							
water	ND	ND	0.1	0.2	**0.4**	**0.4**	0.3
sediment	ND	0.1	0.4	0.8	2.0	5.2	**7.2**
total system	ND	0.1	0.5	0.9	2.4	5.6	**7.5**

1: cold and reflux combined
ND: below limit of detection

Metabolite NOA 448111 was not detected, NOA 448112 and NOA 426289 were < 1 % of AR in water and sediment.

DT_{50}- and DT_{90}-values as estimated by the author are presented in the following table.

Table: DT_{50} and DT_{90}-values for avermectin B_{1a} and metabolites.

System	Conditions	Compound	Compartment	DT_{50} [d]	DT_{90} [d]
sandy loam (River)	aerobic	avermectin B_{1a}	water	0.7	13.5
			sediment	86.4	287.1[1]
			total system	95.7	317.8[1]
sandy loam (River)	aerobic	NOA 426289	sediment	90.0	299.1[1]
			total system	69.7	231.5[1]
sandy loam (River)	anaerobic	avermectin B_{1a}	water	3.0	77.9
			sediment	191.4[1]	635.7[1]
			total system	231.8[1]	769.9[1]
silty clay loam (Pond)	aerobic	avermectin B_{1a}	water	1.5	25.1
			sediment	89.5	297.4[1]
			total system	96.5	320.4[1]
silty clay loam (Pond)	aerobic	NOA 426289	sediment	59.7	198.2[1]
			total system	62.7	208.4[1]
silty clay loam (Pond)	anaerobic	avermectin B_{1a}	water	2.9	53.5
			sediment	391.9[1]	1302[1]
			total system	310.2[1]	1031[1]

Remarks by RMS

Two-compartment modelling not accepted. DT_{50}-values for metabolites were not recalculated because of low levels detected and/or because maximum was reached only by the end of the study. DT_{50}-values for avermectin B_{1a} were recalculated by non-linear fit of first order kinetics. Highest amounts in sediment were taken as starting point for calculation of $DT_{50,sediment}$, calculation for anaerobic incubations not possible because too few data points are available. The results are given in the following table

Table: Recalculated DT_{50}-values for avermectin B_{1a}.

System	Conditions	Compartment	DT_{50} [d]	r^2	n
sandy loam (River)	aerobic	water	1.8	0.9531	7
		sediment	87	0.9420	4
		total system	87	0.9649	7
sandy loam (River)	anaerobic	water	7.2	0.8316	7
		sediment	-[1]		
		total system	230[2]	0.9767	7
silty clay loam (Pond)	aerobic	water	2.9	0.9453	7
		sediment	111	0.9870	4
		total system	91	0.9906	7
silty clay loam (Pond)	anaerobic	water	5.6	0.8954	7
		sediment	-[1]		
		total system	312[2]	0.9217	7

1: calculation not possible, too few data points
2: extrapolated value

$DT_{50,water}$ is mainly determined by sorption. The following results are used for risk assessment:

- Aerobic $DT_{50,water}$ 1.8 and 2.9 days (values represent dissipation)
- Aerobic $DT_{50,sediment}$ 87 and 111 days
- Aerobic $DT_{50,system}$ 87 and 91 days
- maximum 82.8 and 78.1 % of AR present as avermectin B_{1a} in sediment (day 14)

Syngenta endpoint(s) in originally submitted Document III A Section 7:
DT50 values given in table further above ("by the author")

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*
Materials and Methods	[redacted]
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[redacted]

COMMENTS FROM ...

Date
Materials and Methods
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

		Official use only
Reference point (location) in dossier	**7.1.2.2.2/02**	
Title:	Determination of the Aerobic Aquatic Biotransformation of Avermectin B_{1a} Following BBA Guidelines	
Project/Report number:	458-96	
Author(s):	Moore, P.	
Date of report:	25/11/1998	
Published:	Not published	
Testing facility:	Novartis Crop Protection, Greensboro, USA	
Study dates	20/11/1996 to 30/10/1998	
GLP:	Yes	
Reliability indicator	1	X

Reference/notifier	:	Moore, P. (1998)	GLP statement	:	yes
Type of study	:	water/sediment degradation	Guideline	:	BBA IV, 5-1; US-EPA Subdivision N, Section 162-3 and 162-4
Year of execution	:	1996-1998	Acceptability	:	not acceptable
Test substance	:	[5-^{3}H]-avermectin B_{1a}, [redacted]			
		[25-^{14}C]-avermectin B_{1a}, [redacted]			

Substance	Sediment type	Conditions	Ratio sediment water [% v/v]	T [°C]	pH	OM [%]	Duration [d]	Degradation at end [%]	DT_{50} water [d]	DT_{50} sediment [d]	DT_{50} system [d]
^{3}H-avermectin B_{1a}	loam	aerobic	25	20	5.8 - 7.4	6.7	100				
^{14}C-avermectin B_{1a}	loam	aerobic	25	20	5.8 - 7.4	6.7	100				
^{3}H-avermectin B_{1a}	sand	aerobic	25	20	5.1 - 7.6	0.3	100				
^{14}C-avermectin B_{1a}	sand	aerobic	25	20	5.1 - 7.6	0.3	100				

Description

Water/sediment systems. Two types of aerobic water sediment systems were set up and dosed with ^{3}H-avermectin B_{1a} to determine degradation kinetics or ^{14}C-avermectin B_{1a} for isolation and purification of metabolites.
Loam. North Dakota river sediment from Grand Forks County (North Dakota, USA): 50 g dwt sediment; CEC 343 mmol/kg, 3.9 % OC, pH 7.6 for sediment, 8.3 for water. Microbial biomass sediment 323 mg C/kg at start of test, plate counts of water 5.4 x 10^5/mL.
Sand. Wareham river sediment from Plymouth County (Massachusetts, USA): 134 g dwt sediment; CEC 19 mmol/kg, 0.1 % OC, pH 6.9 for sediment, 6.0 for water. Microbial biomass sediment 19.6 mg C/kg at start of test, plate counts of water 2.0 x 10^5/mL.
Sediment wet sieved, 2 mm, corresponding water passed through 0.212 mm. Incubation flasks filled with 2 cm wet sediment and 6 cm corresponding water (234 mL; sediment/water volume ratio 1:3). Additional sterile vessels prepared by steam or chemical sterilisation (NaN_3 or $HgCl_2$). Equilibration for one month at 20 ± 2 °C in the dark under ventilation with air.
Application, incubation and sampling. ^{3}H-avermectin B_{1a} was applied as a solution in methanol (final 0.1 % v/v) into the water phase, final concentration 9 µg/L. For isolation of metabolites, systems were applied with ^{14}C-avermectin B_{1a} in methanol at 450 µg/L. A reference control with ^{14}C-glucose was included (25 mg/L).
Incubation at 20 ± 2 °C in the dark. Water and sediment were sampled in duplicate on t = 0 and after 6 hours and 1 day (only ^{3}H), and 2, 7, 14, 30 and 100 days (both labels), pH, redox potential and oxygen content were recorded on sampling days. Volatiles trapped in ethylene glycol and 10 % KOH (^{14}C-treatment; no traps for ^{3}H).
Sterile incubation checked by plate counts during test.
Chemical analysis.
Water. Radioactivity directly determined by LSC. Extraction two times with methylene chloride followed by acidification of the aqueous phase with HCl and re-extraction with methylene chloride. Methylene chloride extracts were pooled and analysed by 2D-TLC and/or HPLC-UV (245 nm).
Sediment. Total radioactivity analysed by LSC after combustion. Extractions: Step 1. Three times by shaking with methanol/water (1/1 v/v) containing 5 mM ammonium acetate. Extracts combined after centrifugation and analysed by LSC. Step 2: Sediment further extracted two times with ethyl acetate saturated with NH_4OH, extracts pooled and counted by LSC. Extracts of Step 1 and 2 concentrated and analysed by 2D-TLC and/or HPLC, either combined or individually. Bound residues analysed by LSC after combustion. Parent and 8a-hydroxy-avermectin B_{1a} (NOA 488112) isolated from sediment extracts (Step 1) of day-100 loam samples by TLC, and submitted to MS.
Volatiles. Radioactivity in trapping solutions determined directly by LSC.
Glassware. After removal of all fractions, glassware was rinsed with methanol and methanol washes were analysed by LSC.
Reference compounds: abamectin, 8a-oxo-avermectin B_{1a} (NOA 448111), 8a-hydroxy-avermectin B_{1a} (NOA 448112), mono-saccharide of avermectin B_{1a} (NOV 419150) and aglycone of avermectin B_{1a} (NOV 419153).
Calculations. Degradation of avermectin B_{1a} estimated by non-linear fit of first order kinetics.

Results

Both systems generated > 60 % CO_2 from glucose, which demonstrates viability. Loam: Redox potential sediment -25.2 - 134 mV. DO 1.0 - 5.4 mg/L, pH 5.8 - 7.4. Sand: Redox potential sediment 27.2 - 144 mV. DO 2.9 - 6.2 mg/L, pH 5.1 - 7.6.
^{3}H-label. Distribution of radioactivity for the ^{3}H-avermectin B_{1a} incubations is given in the table below for loam and sand systems.

Table: Distribution of radioactivity in loam (North Dakota) and sand (Wareham) system after incubation with ^{3}H-avermectin B_{1a}. All values represent % of AR.

System	Time	Total radioactivity[1]				Water[2]		Sediment[2]		Avermectin B_{1a}[2]	
	[d]	Water	Sediment	Methanol	Recovery	Extract	Remaining	Extract	Bound	Water extract	Sediment extract
loam	0	76.7	12.9	14.0	103.6	14.9	1.5	13.9	0.6	13.9	9.6/14.4[3]
	0.25	61.8	13.0	24.5	99.3	14.0	1.5	8.8	0.2	15.2/9.5[3]	7.9
	1	55.0	18.1	13.5	86.5	14.2	1.6	9.1	0.6	3.9/22.0[3]	7.9
	2	78.5	16.8	7.7	103.0	7.7	1.4	19.8	0.7	6.6[4]	16.9[4]
	7	26.9	78.6	8.7	114.1	1.8	1.5	59.1	2.1	1.1	53.8
	14	21.4	74.7	7.9	104.0	2.0	2.4	65.1	3.5	0.8	53.8
	30	11.6	90.5	4.0	106.0	1.8	2.1	86.8	4.0	0.3	66.0
	61	9.4	86.9	3.7	100.0	0.4	3.9	80.2	6.7	0.0	63.9/41.5[3]
	100	7.1	94.5	4.9	106.4	1.9	4.2	84.9	7.4	0.1	34.8[4]
sand	0	65.8	24.2	15.2	105.2	12.3	2.9	23.8	0.6	10.2	20.0
	0.25	76.3	22.7	12.3	111.2	14.4	1.0	19.0	0.5	13.2	15.9
	1	60.0	27.4	17.4	104.7	16.6	6.8	19.1	1.1	1.2/16.7[3]	13.2
	2	57.7	41.4	12.7	111.7	6.3	7.0	33.8	1.7	2.1/0.3[3]	14.5/24.4[3]
	7	52.0	45.7	6.1	103.8	2.6	1.9	41.3	1.4	1.7[4]	32.4[4]
	14	47.4	60.2	6.8	114.3	9.2	7.2	50.0	3.4	1.0	15.2/33.4[3]
	30	45.4	68.1	5.3	118.8	16.1	10.5	52.9	4.0	2.6	26.9
	61	49.3	54.8	4.6	108.7	12.0	23.4	42.0	6.3	0.6	0.8/1.5[3]
	100	44.0	48.1	8.6	100.7	9.4	24.8	56.6	8.7	1.2/0.0[3]	15.4

1: determined during in-life phase at Springborn
2: determined after shipping by Novartis
3: comment RMS: large difference between duplicates (CV 28 - 120 %), single values given
4: single sample

^{14}C-label. Distribution of radioactivity for the ^{14}C-avermectin B_{1a} incubations is given in the table below for loam and sand systems.

Table: Distribution of radioactivity in loam (North Dakota) and sand (Wareham) system after incubation with ^{14}C-avermectin B_{1a}. All values represent % of AR.

System	Time	Total radioactivity[1]					Water[2]		Sediment[2]		Avermectin B_{1a}[2]	
	[d]	Water	Sediment	Methanol	Volatiles	Recovery	Extract	Remaining	Extract	Bound	Water extract	Sediment extract
loam	0	89.5	3.9	8.2	0.0	101.6	63.3	0.5	5.0	0.4	28.3	4.2
	2	64.4	31.3	6.8	0.0	102.5	32.6	0.4	25.8	1.0	31.1	21.0
	7	30.6	61.5	8.6	0.05	100.7	8.7	0.3	52.9	1.6	7.6	43.1
	14	23.6	70.6	7.4	0.13	101.7	4.5	0.8	63.9	2.1	4.4/1.8[3]	53.9
	30	23.9	82.4	4.0	0.35	110.6	5.7	0.6	79.6	3.9	2.7/1.6[3]	63.0
	61	12.9	85.4	2.6	1.7	103.0	4.0	0.8	72.9	10.2	0.4	47.5
	100	14.1	83.2	3.0	3.0	103.4	9.8	1.9	54.6	17.3	1.0[4]	31.0
sand	0	83.5	8.1	5.6	0.0	97.2	53.4	2.7	21.0	0.6	27.2/50.4[3]	14.1
	2	65.2	27.5	6.3	0.04	98.9	31.8	2.2	28.8	1.9	33.3/20.5[3]	22.8/15.1[3]
	7	51.7	68.6	7.6	0.13	128.0	53.8	0.7	37.1	2.0	45.9	24.5
	14	44.4	42.5	5.8	0.36	93.0	23.4	2.2	48.1	3.5	11.6/6.8[3]	21.1/42.1[3]
	30	51.6	49.7	3.3	2.0	106.6	35.4	7.9	33.2	11.4	3.8/1.5[3]	14.7/2.9[3]
	61	33.0	48.6	3.3	6.9	91.7	16.5	5.6	37.2	17.1	1.1	12.4
	100	28.4	50.5	2.7	9.5	91.1	17.6	4.6	31.7	18.4	0.9/5.0[3]	10.5

1: determined during in-life phase at Springborn
2: determined after shipping by Novartis
3: comment RMS: large difference between duplicates (CV 28 - 120 %), single values given
4: single sample

NOA 448111, NOA 448112, mono-saccharide of avermectin B_{1a} (NOV 419150) and aglycone of avermectin B_{1a} (NOV 419153) were all detected (data of selected replicates were presented in figures only). Author calculated DT_{50} for water and system based on avermectin B_{1a} in extracts and "leaving out selected data points". A second calculation method is provided in which 1) the amount of avermectin B_{1a} in the water extracts is corrected to account for the difference in recovered radioactivity before and after extraction; 2) the amount of radioactivity in the methanol washes was added to the water figures for days 0 to 2, because radioactivity in methanol was accounted for by unchanged avermectin B_{1a} up to day 2 as demonstrated by chromatography of selected samples; 3) the DT_{50} for the total system is estimated using two-phase first order kinetics. Results are given in the table below.

Table: DT_{50}-values as calculated by author.

System	Label	Compartment	DT_{50}	r^2	n	Notes of RMS evaluator
loam	^3H	water	1.9	0.997	7	first-order kinetics; day 1 omitted
		water	2.9	0.992	7	corrected values; day 30 omitted
		total system	78.0	0.989	3	days 0 - 14 omitted
		total system	52.7	0.551	7	day 7 omitted
sand	^3H	water	1.2	0.866	7	day 7 omitted
		water	1.3	0.908	8	corrected values
		total system	52.7	0.551	7	day 7 omitted
		total system	0.78 (1^{st} phase) 52.7 (2^{nd} phase)	0.870	7	corrected values; two-phase first-order kinetics
loam	^{14}C	water	2.3	0.996	7	
		water	4.0	0.998	6	corrected values; day 30 omitted
		total system	118	0.953	4	days 2, 7, 30 omitted
		total system	2.4 (1^{st} phase) 90.7 (2^{nd} phase)	0.996	6	corrected values; two-phase first-order kinetics; day 30 omitted
sand	^{14}C	water	6.5	0.980	6	day 7 omitted
		water	6.9	0.996	5	corrected values; days 7 and 30 omitted
		total system	26.7	0.840	6	day 7 omitted
		total system	14.6 (1^{st} phase) 112 (2^{nd} phase)	1.0	5	corrected values; two-phase first-order kinetics; days 7 and 30 omitted

Remarks by RMS

Amount of sediment given as 50 and 134 g wet weight in summary report, but refer to dry weight according to original report on in-life phase of study. Corresponding wet weight not given, sediment/water ratio cannot be calculated.

LSC analysis of water, sediment and methanol is performed during in-life phase, by Springborn Laboratories. New LSC, further extraction and analysis was performed by Merck and re-analysis was done by Novartis (after acquisition of abamectin) with slightly different procedures. Methods of both Merck and Novartis are reported, but it is not clear to which method the final figures refer. It is assumed that Novartis' results are presented, and therefore only Novartis' extraction and analysis method is described.

For water, sum of extractable and remaining radioactivity is much lower than total radioactivity as counted by direct LSC at Springborn laboratories. Difference is not due to extraction efficiency, which is 89.8 % for water and 112 % for sediment (determined for ^{14}C). Loss of radioactivity during shipping and storage is not likely, as figures for sediment before and after extraction are more or less consistent with each other. Authors apply correction to the data of the water extracts, but no explanation for the difference is given.

Large differences in extractable avermectin B_{1a} between replicates on various occasions. Mean values are used by authors, other values are arbitrarily omitted from DT_{50}-calculation because they do not fit into the degradation pattern. Data for metabolites not given in main text, values as presented by notifier in document MIII, section 5, are taken directly from figures with TLC-counts and refer to selected single replicates. In view of the above, the study is considered not acceptable for risk assessment.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date **Materials and Methods** **Results and discussion** **Conclusion**	EVALUATION BY RAPPORTEUR MEMBER STATE *24-10-2007* [redacted]

Reliability	[redacted]
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

		Official use only
Reference point (location) in dossier	**7.1.2.2.2/03**	
Title:	Degradation of Abamectin in a Field Study Simulating both Drift and Runoff	
Project/Report number:	ENC-2	
Author(s):	Ku, C.C., Wislocki, P. and Lu, A.	
Date of report:	23/09/1986	
Published:	Not published	
Testing facility:	Merck Sharp & Dohme Research Laboratories, Three Bridges, New Jersey, USA	
Study dates	Not applicable	
GLP:	Yes	
Reliability indicator	1	X

Reference/notifier	:	Ku, CC, Wislocki, P. and Lu, A. (1986)	GLP statement	:	yes
Type of study	:	outdoor semi-field water/sediment degradation	Guideline	:	in-house protocol
Year of execution	:	1985	Acceptability	:	not acceptable
Test substance	:	Abamectin 0.15 EC, batch [redacted], purity not specified, clear liquid			

Substance	Location	Application type	Time of application	T [°C]	Sediment type	Ratio sediment water [g/mL]	pH	OM [%]	Duration [d]	DT_{50} water [h]	DT_{50} system [h]
Abamectin 0.15 EC	St. Michaels, MD, USA	spray, 0.112 kg as/ha	August	18 - 32	sandy clay loam	0.14	4.6 - 8.0	0.3	52		15

Description

Outdoor water/sediment systems were used to estimate fate and behaviour of abamectin simulating exposure by drift or by run-off from adjacent treated soil.

Water/sediment systems. Galvanised, epoxy coated tanks (L 2.4, W 0.9, D 0.6 m) were buried 0.3 m deep into the ground at the test site of Wildlife International in St. Michaels, Maryland, USA. A 2.5 cm layer of clay loam

soil (10.1 % OM, CEC 109 mmol/kg, pH 5.7) was put on the bottom, a 5 cm layer of a sandy clay loam soil (0.3 % OM, CEC 109 mmol/kg, pH 5.0) was applied evenly over the clay layer and ca. 1135 L water from a farm pond was added. Systems were allowed to settle for one week.

Application, incubation and sampling.

Simulated drift. Three systems were sprayed with a slide sprayer at application rates of 0.0112, 0.0336 and 0.112 kg as/ha on August 12, 1985. One tank per treatment, one untreated control.

Simulated run-off. Sandy clay loam soil was sieved (ca. 7.9 mm) and spread out on foil in a 3 mm layer and sprayed at 0.028, 0.084 or 0.28 kg as/ha. Treated soil was added to the tanks by broadcast application in small portions within one hour after spraying (non-aged run-off; August 12, 1985) or after 90 hours of ageing (aged run-off; August 16, 1985), one tank per treatment, ca. 6.5 L treated soil per tank (ca. 0.3 cm). One control with untreated soil.

Sampling. Treated soil was sampled before introduction to the tanks, and during ageing. Water and sediment samples were taken immediately after application and after 6 hours (water only) and 1 day and after 4, 8, 15, 31 and 52 days (drift and non-aged run-off) or 3, 7, 14, 30 and 51 days after addition of the treated soil (aged- run-off). Water samples taken at three depths, mid-level analysed. Sediment was sampled with 46.5 mm wide corers (20 or 61 cm long), same time points as for water, except for first sampling occasion. Samples were frozen. Tanks were covered with plastic tarps until one day after treatment to prevent initial photolysis, and thereafter each evening and when rain was forecasted. Physico-chemical parameters of water were measured at the start and end of the study.

Chemical analysis.

Water according to Merck method "LC-fluorescence for Avermectin B_1 (MK-0936) in water, August 18, 1983", no separation of suspended particles. Sediment according to Merck method 8001: HPLC fluorescence determination of Avermectin b_1 in pond water and sediment, February 7, 1986. Soil according to Merck method 3005: LC-Fluorescence assay for Avermectin B_1 (MK-0936) in soil, October 1, 1984. LOD 0.1 µg/L for water and 1 µg/kg for soil.

Recoveries as reported in the different analysis reports: 112 % (n = 4), 70 - 90 % (average 77%, n = 4) and 75 - 90 % (average 81 %, n = 4) for water; 60 % (n = 3) and 97 - 110 (average 95 %, n = 4) for sediment; 70 - 103 % (average 85 %, n = 4) and 67 - 94 % (average 81 %, n = 5) for soil.

Results

Water temperature during test 17.5 - 31.5 °C, DO 4.6 - 11.2 mg/L, pH 4.6 - 8.0. On August 18 (six days after application for drift and non-aged run-off; two days for aged run-off), heavy rain and wind associated with a hurricane, caused increased water levels in nine of the tanks. Estimated increase, measured by the height of algal growth, was 17.7 to 70.9 L (1.6 - 6.2 % of total initial water volume). Concentrations in treated soil after spraying are given in the table below, concentrations in water and sediment after incubation are given in the table further below. Residues in water and sediment of control systems were below LOD.

Table: Concentrations of abamectin in soil for run-off treatment after spraying

Treatment for which prepared	Application rate [kg as/ha]	Nominal concentration[1] [µg/kg]	Ageing time [d]	Concentration [µg/kg]	% of nominal
run-off, non-aged	0.028	622	0	1067	172
	0.084	1867	0	1374[2]	74
	0.28	6222	0	1193	19
run-off, aged	0.028	622	0	174	28
			0.25	24	
			2	1.8	
			4	2.4	
run-off, aged	0.084	1667	0	794	43
			0.25	218	
			2	< 1	
			4	< 1	
run-off, aged	0.28	6222	0	3236[2]	52
			0.25	186	
			2	56[2]	
			4	16	

1: calculated by RMS from application rate, soil layer depth 0.003 m and default soil bulk density 1500 kg/m^3

2: values reported as avermectin B_{1a} in analysis report, as abamectin in main report

Table: Concentrations of abamectin in water and sediment after incubation in outdoor tanks

Way of treatment	Application rate [kg as/ha]	Compartment	Measured concentrations 0	0.25	1	3/4	7/8	14/15	30/31	51/52	d
drift	0.0112	water	1.03	0.24	< 0.1	< 0.1	< 0.1	0.11	< 0.1	< 0.1	µg/L
		sediment	< 1	-	-	< 1	< 1	< 1	< 1	< 1	µg/kg
	0.0336	water	12.5	0.85	0.17[1]	0.11	< 0.1	< 0.1	< 0.1	< 0.1	µg/L
		sediment	1.9	-	-	< 1	< 1	< 1	< 1	2.8	µg/kg
	0.112	water	13.7	1.05[1]	0.73	0.77	0.29	0.10	< 0.1	< 0.1	µg/L
		sediment	2.89	-	3.48[1,2]	2.71[1]	3.11	1.69[1]	1.7	< 1	µg/kg
run-off, non-aged	0.028	water	0.20	< 0.1	0.10	0.52	0.10	0.11	< 0.1	< 0.1	µg/L
		sediment	1.7	-	3.3	7.3	3.8	4.7	1.4	2.7	µg/kg
	0.084	water	0.25[1]	0.43[1]	0.26	0.46	0.24	0.35	0.1	< 0.1	µg/L
		sediment	2.81[1,2]	-	18.8[1]	1.88[1]	15.1[1]	21.7[1]	12.9	10.6	µg/kg
	0.28	water	3.52	0.78	3.52	3.52	3.68	1.28	0.24	< 0.1	µg/L
		sediment	14.0	-	29.3	21.4	6.6	22.8	9.5	6.1	µg/kg
run-off, aged	0.028	water	< 0.1	< 0.1	< 0.1						µg/L
		sediment	< 1	-	< 1						µg/kg
	0.084	water	< 0.1	< 0.1	< 0.1						µg/L
		sediment	< 1	-	< 1						µg/kg
	0.28	water	< 0.1	< 0.1	< 0.1						µg/L
		sediment	< 1	-	<1	1.42	< 1	< 1	< 1	< 1	µg/kg

1: values reported as avermectin B_{1a} in analysis report, as abamectin in main report
2: values taken from analysis report, given as 2.61 and 3.64 in main report

Authors mention half-life of abamectin of ca. 5 - 10 hours in soil, 4 days in water and 2 to 4 weeks in sediment. Based on acute and chronic toxicity data, authors conclude that when 100 % drift occurs at the proposed use rate of 2.8 g as/ha, concentrations that are acute toxicity to macro-invertebrates will be present for a brief period (much less than one day), and levels of chronic toxicity will be present for less than two days.

Remarks by RMS

Pages with physico-chemical data are missing from in-life report, reported figures taken from the text. Analysis results are given in five different reports included as Annexes to the main report, some by mistake copied in reverse order. In these reports, sample codes are given without identification of treatment, and in the main report, no reference is made to the corresponding underlying analysis reports. Most figures could be verified using the sample codes in the raw sampling data sheets, but because some codes were not readable and pages were missing, it was not possible to check all raw data.
Analysis results are given as avermectin B_{1a} or avermectin B_1 (= abamectin) in individual reports, and as abamectin in main report.
To establish the mass balance, the total amount of added abamectin is calculated either from the application rate and the surface area of the tanks (drift treatment) or from the concentration in application soil and the reported amount of soil added (run-off treatment). Amounts in water and sediment on t = 0 and maximum amounts for run-off treatments are calculated from the measured concentrations in water and sediment, assuming a total water volume of 1135 L and a total sediment weight of 172 kg (total sediment layer depth 5.3 cm, surface area 2.16 m^2 and bulk density 1000 kg/m^3). The resulting figures are given in the table below.

Table: Mass balance on t = 0

Way of treatment	Application rate [kg as/ha]	Added amount of abamectin [µg]	Recovery on t = 0 as percentage of applied water	sediment	total	Maximum recovery as percentage of applied water	sediment	total	on day
drift	0.0112	2420[1]	48	-	48				
	0.0336	7258[1]	195	4.5	200				
	0.112	24200[1]	64	2.1	66				
run-off, non-aged	0.028	9363[2]	2.4	3.1	5.5	6.3	13	30[3]	3
	0.084	11777[2]	2.4	4.1	6.5	3.4	32	35	14/15
	0.28	10098[2]	40	24	64	40	50	90	1
run-off, aged	0.028	20.3[2]	-	-	-	-	-	-	
	0.084	< 8[2]	-	-	-	-	-	-	
	0.28	135[2]	-	-	-	-	181	-	3/4

1: calculated from application rate and surface area of tanks (2.16 m^2)
2: calculated from measured concentration in non-aged or aged application soil (see the first table under Results above), and reported added soil weight for each treatment (8426 - 8775 g)
3: difference due to rounding-off

Calculations show that the total recovered amount on t = 0 deviated strongly from the nominal applied amount. The variable recovery in the water phase might be due to the fact that suspended particles were included in the water analysis. For the non-aged run-off treatment, the highest recovery is found later on, indicating that settlement of contaminated soil took some time. Concentrations are dependent on settlement rate, adsorption, degradation and formation of bound residues. Because it is not possible to disentangle these processes, it is not possible to derive reliable estimates of DT_{50} values. The study is considered not acceptable for risk assessment.

Syngenta findings in originally submitted Document III A Section 7:
Under simulated spray-drift deposition conditions, abamectin rapidly distributed between the water and sediment phases. Between the zero-hour and the 19 hour sampling the levels of abamectin in the water-phase decreased by 77 to 93% of the applied dose. After this initial distribution and rapid degradation the level of abamectin in water decreased with a half-life of approximately 4 days. Abamectin decreased in the sediment samples from both the drift and the runoff scenarios with a half-life of approximately 2 to 4 weeks, although there was variation in the sediment concentrations.

Results of analyses from the runoff phase of the experiment indicated that abamectin, sprayed onto soil and exposed to sunlight, underwent rapid degradation with a half-life of between 5 and 10 hours. Soil which had been treated and aged prior to being added to the water tanks therefore contained only low levels of abamectin, and, when added to the tanks did not result in detectable levels in the water phase. The addition of soil to the water tanks immediately after spraying with abamectin resulted in detectable levels in both the water and the sediment. Residue levels found in the sediment were equivalent to 0.33 µg/kg at the 1 x level. Equilibration between the sediment and the water occurred over a longer period of time than in the drift phase of the experiment.

In water, the half-life of the abamectin following equilibration was 4 to 7 days. The half-life in sediment could not be determined due to the uneven distribution of the soil onto the sediment in the tank.

Despite the conservative approach taken (i.e. exclusion of light from the test systems using tarpaulin covers over the initial 14 to 18 hour period following treatment) the abamectin levels in sediment and water were low and demonstrate that under field conditions exposure of- and impact to- aquatic ecosystems from abamectin via drift or runoff will be significantly reduced.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*
Materials and Methods	
Results and discussion	

Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

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Reference point (location) in dossier	**7.1.2.2.2/04**	
Title:	Assessment of the potential biological effects of Abamectin MK936, 018 EC (A8612A) exposures on aquatic ecosystems as measured in an outdoor microcosm m tank system	
Project/Report number:	982570	
Author(s):	Rufli, H.	
Date of report:	20/12/1999	
Published:	Not published	
Testing facility:	Novartis Crop Protection AG, Ecotoxicology Department, Basle, Switzerland	
Study dates	Not applicable	
GLP:	Yes	
Reliability indicator	1	X

Reference/notifier	:	Rufli, H. (1999)	GLP statement	:	yes
Type of study	:	outdoor microcosm study	Guideline	:	Monks Wood workshop, 1991; Wintergreen Workshop, 1991; EWOFFT, 1992; draft OECD (issued by SETAC, 1993)
Year of execution	:	1998 - 1999	Acceptability	:	acceptable
Test substance	:	A 8612A (Vertimec EC 018), batch [redacted] purity 19.5 g abamectin/L, appearance light yellow liquid			

Substance	Location	Application type	Time of application	T [°C]	Sediment type	Ratio sediment water [g dwt/mL]	pH	OM [%]	Duration [d]	DT_{50} water [d]
A 8612A	Stein, CH	spray	June	21	sandy loam[1]	0.09[2]	7.2	2.7[1]	91	9.6[3]

1: based on average of 24 tanks
2: assuming layer depth 10 cm, bulk density 1300 kg/m^3 and water volume 10000 L
3: DT_{50} represents dissipation by sorption and degradation

Description

Outdoor microcosm study summarised in Document IIIA reference point 7.4.3/01. Distribution and fate is evaluated here.
Microcosms. Polyethylene tanks (depth 1.5 m, diameter 3 m, volume 10 m^3), located at the test site of Syngenta in Stein, Aargau, CH, had been established in Spring 1996 and used for experiments since then. Sediment type sandy loam, layer depth ca. 10 cm at time of construction, on a 5 cm clay layer. Algae, zooplankton and other organisms had been introduced along with the water and sediment, macrophytes had been planted in March 1997 (*Myriophyllum verticullatum*) and March 1998 (*Potamogeton crispus*). Cosms were set up for the present experiment 3 months before application, water was circulated from a supply pond until one day before application and recirculation was started again 14 days after application.
Application, concentrations, replicates. Test substance diluted in double distilled water and sprayed on the water surface in a single application on June 30, 1998. Six dose levels, 3.3, 10. 31. 92, 278 and 833 µg product/L, equivalent to 0.066, 0.20, 0.62, 1.8, 5.6 and 17 µg as/L. Three replicate tanks per dose and three controls.
Chemical analysis. Water and sediment samples were taken from the control and highest concentration 7 days before application and at regular time intervals after application, additional sampling from all tanks 2 hours after application. Water: according to analytical method AM98-07. Water samples were concentrated by SPE, columns were eluted with acetonitrile. Eluate was made up to volume with bidistilled water and analysed by HPLC-UV (245 nm). LOQ 0.1 µg/L. Application solutions were analysed according to method AM98-07a without pre-concentration, LOQ 0.5 µg/L. Sediment: according to method AM99-03. Sediment and interstitial water were separated by centrifugation. Water was cleaned up by SPE, elution with water/acetonitrile 7/3 (v/v), analysis by HPLC-UV (245 nm). Sediment was extracted by shaking with methanol, extracts were diluted with water and cleaned up by SPE, analysis by HPLC. LOQ for sediment 0.02 mg/kg, for interstitial water 0.2 mg/L.

Results

Conditions. Day of application: wind speed 0.5 - 4.5 m/s, 24.5 °C at 13.00 h, no rain. Water temperature during test period 14 - 27 °C, overall average in control 20.6 °C. DO 105 - 260 % saturation from day 0 - 28, 50 - 246 % from day 35 - 91. The pH was between 7.5 and 9.8.
Chemical analysis. Analytical recovery for abamectin in water 59.4 - 110 %, average 89.6 % (n = 5, RSD 22.3 %). For sediment, recovery was 44.8 - 152.4 %, average 82.4 % (n = 8, RSD 47.3 %). All measured concentrations were corrected for average recovery. Concentrations in control were always < LOQ.
Concentrations two hours after application were < LOQ at test concentration 0.066 µg as/L, and on average 99, 84, 54, 55 and 53 % of nominal at test concentrations 0.20, 0.62, 1.8, 5.6 and 17 µg as/L, respectively.
Measured concentrations in water and sediment of the highest test concentration (17 µg as/L) during the test are given in the table below. Concentrations in interstitial water were always < LOQ.

Table: Measured concentrations in dose level 17 µg as/L (240 g as/ha)

	measured concentrations of abamectin					
	water [µg/L]			sediment [µg/kg dwt]		
Time	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
2 h	7.00	14.6	4.74	58.4	72.5	49.4
1	12.7	10.4	10.6			
3	9.45	10.1	9.49			
6	7.83	7.87	8.60	< LOQ	23.1	< LOQ
13	5.46	6.18	4.56	< LOQ	< LOQ	< LOQ
21	0.858	0.844	0.853			
28	0.201	0.234	0.208	< LOQ	< LOQ	< LOQ
35	< LOQ	< LOQ	0.100			
49	0.234	< LOQ	< LOQ			

Author estimates DT_{50} as 5 days.

Remarks by RMS

According to the study plan, information on previous experiments performed with other products in the microcosms, should be supplied in the report. The report refers to the raw data, but this information is missing. RSD of analytical methods is too high, but average recovery is acceptable. Highest dose level corresponds with 240 g as/ha. Author reported total recovery just after application as 83 %, 69 % in water and 14 % in sediment. Figure of 69 % is average of dose levels 0.20 - 17 µg as/L, figure of 14 % must originate from dose level 17 µg/L, as this is the only treatment where sediment is analysed. The figure, however, cannot be deduced from the analysis data. With a 10-cm sediment layer, a surface area of 7 m^2 and assuming a dry bulk density of 1500 kg/m^3, each tank contains 1050 kg dwt sediment. From the measured concentration in sediment, the amount of abamectin is calculated as 53144, 65975 and 44954 µg for tank 1, 2 and 3, respectively. This is 32, 39 and 37 % of the nominal applied amount of 167461 µg per tank (8.33 g product, 19.46 g as/L, density 0.968 g/mL). Assuming 10000 L water per tank, corresponding figures for water are 70000, 146000 and 47400 µg, equivalent to 42, 87 and 28 % of the applied amount. The mass balance for the highest dose level is thus 64, 126 and 65 % (average 85 %), mass balances for the other levels cannot be calculated. Metabolites were not analysed. $DT_{50,water}$ for dose level 17 µg as/L is recalculated by non-linear fit of first order kinetics. Using all time points, resulting $DT_{50,water}$ for tank 1, 2 and 3 are 10.0 days (r^2 0.8195), 7.9 days (r^2 0.9312) and 10.9 days (r^2 0.7079), average is 9.6 days. Recirculation of water was started 14 days after application, which can explain the sudden drop in concentrations between day 13 and 21. $DT_{50,water}$ values calculated using data of days 0 or 1 to 13, however, are similar to those calculated for the whole period. The low recovery on the first sampling point (53 %), together with substantial amounts in sediment, indicates that initial sorption may have taken place. The result $DT_{50,water}$ 9.6 days is considered for risk assessment.

Syngenta endpoint(s) in originally submitted Document III A Section 7:
water-phase DT_{50} for abamectin of approximately 5 days.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*24-10-2007*
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

		Official use only
Reference point (location) in dossier	**7.1.2.2.2/05**	
Title:	Assessment of the Effects of Abamectin 018 EC (A8612A) in Outdoor Microcosms	
Project/Report number:	2002590	
Author(s):	Knauer, K.	
Date of report:	19/04/2002	
Published:	Not published	
Testing facility:	Not applicable	
Study dates	Not applicable	
GLP:	Yes	
Reliability indicator	1	X

Reference/notifier	:	Knauer, K. (2002)	GLP statement	:	yes
Type of study	:	outdoor microcosm study	Guideline	:	Monks Wood workshop, 1991; EWOFFT, 1992; draft OECD (issued by SETAC, 1993); HARAP, 1998; CLASSIC, 1999
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	A 8612A (Vertimec 018 EC), batch [redacted] purity 19.6 g abamectin/L, appearance yellow to red brown liquid			

Substance	Location	Application type	Time of application	T [°C]	Sediment type	Ratio sediment water [g dwt/mL]	pH	OM [%]	Duration [d]	DT_{50} water [d]
A 8612A	Stein, CH	spray, 3 x 17 g as/ha	May	18	loam[1]	0.11[2]	7.3	5.5[1]	91	5.2[3]

1: based on average of 24 tanks
2: assuming layer depth 10 cm, bulk density 1500 kg/m^3 and water volume 10000 L
3: DT_{50} represents dissipation by sorption and degradation

Description

Outdoor microcosm study summarised in Document IIIA reference point 7.4.3/02. Distribution and fate is evaluated here.

Microcosms. Polyethylene tanks (depth 1.5 m, diameter 3 m, volume 10 m^3), located at the test site of Syngenta in Stein, Aargau, CH, had been established in Spring 1996 and used for experiments since then. Sediment type loam, layer depth ca. 10 cm at time of construction, on a 5 cm clay layer. Algae, zooplankton and other organisms had been introduced along with the water, most dominant macrophyte was *Elodea canadensis*, *Myriophyllum verticullatum* and *Potamogeton crispus* were present at lower abundances. Water was exchanged on March 8, 2000 (1.5 months before application), sediment was not replaced but did not contain residues of the product and metabolites tested in 1999. Water circulation was started on March 21, 2000, and stopped on the day of first application (May 9, 2000).

Application, incubation and sampling.

Test substance diluted in double distilled water and sprayed on the water surface three times with a 7-days interval, starting on May 9, 2000. Six dose levels, 3.47, 10.4, 31.3, 93.8, 282 and 847 g product/ha, equivalent to 0.071, 0.21, 0.64, 1.9, 5.8 and 17 g as/ha. Assuming complete mixing, application rates are corresponding to 0.245, 0.736, 2.21, 6.63, 19.9 and 59.9 µg product/L or 0.005, 0.015, 0.045, 0.135, 0.405 and 1.22 µg as/L.

Three replicate tanks per dose and three controls. Water samples were taken 7 days before application and at regular time intervals after application. Sediment samples were collected, but not analysed due to technical failure.

Chemical analysis. Dose levels 0.135, 0.405 µg as/L, 1.22 µg/L up to day 29: According to Syngenta analytical method AM2000-07. Aliquots of water samples were diluted with acetonitrile (acetonitrile volume 6 %) and passed over a C_{18}-column. Columns were eluted with acetonitrile, the eluate is made up to volume and analysed by HPLC-UV (245 nm), LOQ 0.1 µg/L (abamectin). Dose levels 0.005 – 0.045 µg as/L, 1.22 µg/L as from day 29: According to Solvias analytical method A.13.S011_2. Water samples had been extracted on SPE columns, concentration factor usually 25, solvent water/acetonitrile 1/1 (v/v). Analysis by HPLC-MS, LOQ 1 ng/L.

Results

Conditions. Water temperature on the day of application was 19.3 - 19.9 °C, temperature ranged from 15.5 to 24.6 °C during the experiment. Overall average temperature in the control was 18.3 °C. DO was > 100 %, pH was between 9.3 and 10.3, no difference between dose levels.

Chemical analysis. Analytical recovery for method AM2000-07 was 87.4 – 144 %, average 104.1 % (n = 4, RSD 25.7 %). Recovery for method A.13.S011_2 was 78 – 133 %, average 104 % (n = 9, RSD 19 %). Measured concentrations of abamectin in control and are given in the table below (dose levels 0.071 - 5.8 g as/ha) and the table further below (control and 17 g as/ha). All values are corrected for recovery.

Table: Measured concentrations of abamectin in outdoor microcosms: dose levels 1 to 5.

Time [days]	Nominal dose level of abamectin [g as/ha] (Corresponding nominal concentration in µg as/L)														
	3 x 0.071[1] (3 x 0.005 µg as/L)			3 x 0.21[1] (3 x 0.015 µg as/L)			3 x 0.64[1] (3 x 0.045 µg as/L)			3 x 1.9[2] (3 x 0.135 µg as/L)			3 x 5.8[2] (3 x 0.405 µg as/L)		
	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
0.25[3]	0.0081	0.0031	0.0058	0.0063	0.0098	0.0060	0.0321	< LOD	< LOD	0.0957	0.0780	0.047	0.207	0.228	0.117
1	0.0057	0.0043	0.0036	0.0081	0.0071	0.0043	0.0206	0.0178	0.0208	0.0907	0.0957	0.0818	0.233	0.282	0.279
7.25[3]	0.0128	< LOD	0.0013	0.0287	< LOD	0.0141	0.0298	0.0049	0.0030	0.125	0.0866	< LOD	0.299	0.322	0.323
8	0.0170	0.0077	0.0165	0.0278	< LOD	0.0246	0.0377	0.0226	0.0395	0.102	0.103	0.0912	0.413	0.306	0.282
14.25[3]	0.0114	0.0061	0.0022	0.0056	0.0071	0.0043	0.0581	0.0316	0.0220	0.120	0.503	0.467	0.527	0.686	0.323
15	0.0072	0.0069	0.0083	0.0260	0.0090	0.0130	0.0368	0.0372	0.0292	0.104	0.134	0.0826	0.436	0.451	0.110

1: Solvias data; 2: Syngenta data; 3: 6 h after application

Table: Measured concentrations of abamectin in outdoor microcosms: control and dose level 6.

Time [days]	Nominal dose level of abamectin [g as/ha] (Corresponding nominal concentration in µg as/L)					
	control[1,2]			3 x 17[1,2] (3 x 1.22 µg as/L)		
	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
0.25[1,3]	0.0018	0.0011	0.0011			
0.25[2,3]	< LOD	< LOD	< LOD	0.603	0.802	0.535
1[2]				0.920	0.980	0.929
3[2]	< LOD			0.592	0.631	0.549
7[2]		< LOD		0.386	0.426	0.282
7.25[2,3]	< LOD	< LOD	< LOD	0.836	1.08	0.790
8[2]			< LOD	1.01	< LOD	0.942
10[2]	< LOD			0.653	0.540	0.537
14[2]			< LOD	0.606	0.452	0.742
14.25[2,3]	< LOD	< LOD	< LOD	1.33	1.96	2.07
15[2]	< LOD			1.36	1.10	1.06
17[2]		< LOD		1.32	1.07	0.852
21[2]			< LOD	0.790		0.970
29[2]	< LOD			0.278	0.217	0.326
29[1]				0.1066	0.1592	0.1875
35[1]				< LOD	0.0579	0.0291
49[1]				0.0785	0.0242	0.0456
63[1]				0.0119	0.0107	0.0122
79[1]				0.0062	0.0075	0.0078
91[1]				0.0033	0.0023	0.0039

1: Solvias data
2: Syngenta data
3: 6 h after application

Author calculated dissipation half-life for water by non-linear regression of first order kinetics, using the data of the highest dose level after the 3rd application and taking an estimated initial concentration of 1.822 µg as/L on day 14, just after the 3rd application as starting point. This initial concentration is the sum of the average

measured concentration on day 14 (0.6 g as/L) and the applied dose (59.9 µg product/L, equivalent to 1.22 µg as/L). The resulting DT_{50} is 4.9 days.

Remarks by RMS

According to the study plan, information on previous experiments performed with other products in the microcosms, should be supplied in the report. The report refers to the raw data section, but there the information is missing. From the description it is likely that the experiment described above (June 1998) was performed in the same tanks as the present experiment, but tanks were not assigned to the similar treatments. Analysis was performed at different laboratories, at Solvias AG, Basel, CH and at Syngenta, Basel, CH. Recovery of Syngenta method is variable. There are differences between analysis results of both labs, see data for dose level 3 x 17 g as/ha, day 29. In the Solvias report it is mentioned that samples were filtered over 0.45 µm, it is not clear whether this was done before or after SPE. Authors use average of three tanks to calculate DT_{50}, but because each tank represents an individual system it is better to estimate the dissipation rate for each tank separately and then calculate the average rate. By using the estimated initial concentration of 1.822 µg/L, authors assume instantaneous distribution of the applied dose, which is not necessarily appropriate in these systems. Applying non-linear regression of first order kinetics on the data from time point 14.25 days onwards and using both day-29 values, DT_{50} values for dissipation from water are 6.5 days for tank 1 (r^2 0.9644), 3.8 days for tank 2 (r^2 0.9450) and 5.3 days for tank 3 (r^2 0.8741), average 5.2 days. Other studies show that dissipation from water is mainly determined by initial sorption. Because sediment and macrophytes are not analysed in the present study, a clear distinction between sorption and degradation cannot be made. The result $DT_{50,water}$ 5.2 days (dissipation) is considered for risk asessment.

Syngenta endpoint(s) in originally submitted Document III A Section 7:
median dissipation time (DT_{50}) was estimated to be 4.9 days (range 4.3 – 5.8 days) [i.e. small difference to RMS endpoint]