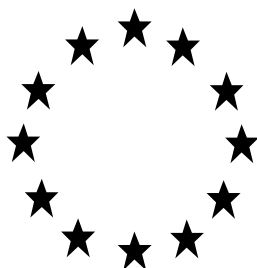


Evaluation of active substances
COMPETENT AUTHORITY REPORT
(submitted by the evaluating Competent Authority)

Addendum



s-Methoprene

Product type 18

(Insecticides)

Evaluating Competent Authority: Ireland

November 2018

Substance Name: S-methoprene

EC Name: isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate

EC Number: 254-993-2

CAS Number: 40596-69-8

Applicant: Bábolna Bio Ltd

4.1.1.2 Biotic degradation

4.1.1.2.1 Biodegradability (ready/inherent)

Summary table - biodegradation studies (ready/inherent)											
Method, Guideline, GLP status, Realibility	Test type ¹	Test parameter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Remarks	Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]		
Modified MITI Test (II), OECD guideline 302C, GLP, The study was considered acceptable with a Reliability score of 1.	Inherent Biodegradability	See below	Activated sludge	100 ppm	None	n/a	30 ppm	28 days	> 70%	See below	Dr. Vértési, A. (2014); Inherent Biodegradability of S-Methoprene In Modified MITI Test (II). TOXI-COOP ZRT, 8230 Balatonfüred, Arácsi út 97, Hungary , unpublished report No.: 484.462.3617

¹ Test on inherent or ready biodegradability according to OECD criteria

The biochemical oxygen demand (BOD) values were measured continuously during the experiment.

- Under the test conditions the percentage biodegradation of S-Methoprene reached a mean of 4.2 % after 7 days, 24.5 % after 14 days, 77.5 % after 21 days and 85.8 % after 28 days based on its ThOD.
- The Inherent biodegradability of S-Methoprene Technical was determined from the BOD measurement over 28 days according to OECD guideline 302C. The percentage of degradation of S-Methoprene Technical was calculated using the BOD method, and supplemental chemical analysis was also carried out using HPLC method with UV detection on a Phenomenex, Luna 3 μ C18 column.

From the BOD method the biodegradability was calculated to be 85.8% and by chemical analysis the degradation was found to be 74.2%. This modified MITI (II) test showed >70% degradation within 28 days. This represents inherent biodegradability (as specified in TGD). The failure to reach 70% within 14 days means that the specific inherent biodegradability criteria were not met and therefore that extrapolation of the results for use in STP models is not possible.

4.1.1.3.2 Biodegradation in freshwater

Water/sediment degradation test

Summary table – fresh water/sediment degradation									
Method, Guideline, GLP status, Reliability	Test type ¹	Exposure	Test system		Test substance concentration	Incubation period	Degradation (DT ₅₀ / days)	Remarks	Reference
			Water	Sediment					
Aerobic transformation in aquatic sediment systems, OECD 308, GLP, The study was considered acceptable with a Reliability score of 1.	Biodegradation in freshwater		River Rhine, Mumpf, AG, Switzerland		0.059mg/kg total system	100 days	<u>Parent</u> total system 2.50 <u>Metabolite M2</u> total system 5.40 <u>Metabolite M3</u> total system 2.29	See below	Gassen, M. (2015), Route and Rate of Degradation of [14C]S-Methoprene in Aerobic Aquatic Sediment Systems. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland, unpublished report no.: D93728.
			Fröschweiher pond, Möhlin, AG/Switzerland				0.061mg/kg total system		

¹ Test according to OECD criteria

* parameter was derived using DFOP kinetics. Comparison of biphasic kinetic parameters with trigger cutoffs is not ideal. Therefore calculation of the DT₅₀ from the slow phase of the degradation yields a more conservative estimate of degradation:

$$DT_{50} = \ln 2 / k_1 = 0.6931 / 0.1089 = 6.4 \text{ days @ } T = 20^\circ\text{C} \text{ or } 12.1 \text{ days @ } T = 12^\circ\text{C}.$$

Data indicated that [14C] S-Methoprene and metabolites degraded at a very rapid rate and showed rapid dissipation from the total system.

- Total recoveries of the applied radioactivity (material balances) averaged $92.7 \pm 7.2\%$ and $96.1 \pm 10.7\%$ of the applied radioactivity for the river and pond systems, respectively.
- After treatment, the majority of the radioactivity applied was detected in the water phase, representing 100.5% and 104.5% of the applied radioactivity for river and pond, respectively. In both systems the level of radioactivity in the water phase rapidly decreased over time, mainly by degradation and adsorption to the sediment. Within two days, the amount in the water phase had decreased to 38.2% and 28.5% of applied radioactivity for river and pond systems respectively. On Day 100, corresponding values were 1.2% and 0.6% of the applied radioactivity for river and pond, respectively.
- The total extractable radioactivity from sediments initially increased reaching maximum mean amounts of 21.2% (river) and 25.5% (pond) of applied after two days. Thereafter, levels decreased and on Day 100, 3.4% and 2.0% of the applied radioactivity was still extractable from the river and pond sediments, respectively. Soxhlet extractions were performed from Day 2 onwards, recovered up to 3.4% (river system, Day 2) and 4.7% (pond system, Day 14) of the applied radioactivity.
- The formation of radioactive carbon dioxide was significant, and constantly increased throughout incubation in both systems, reaching maximum mean amounts of 54.9% (river) and 67.5% (pond) of the applied radioactivity after 100 days of incubation. Organic volatile compounds never exceeded 0.6% of the applied radioactivity in either system.
- The relative amounts of [^{14}C]S-Methoprene and degradation products present in water and the sediment extracts were determined by chromatographic profiling by normal phase TLC.
- Significant metabolites in the water phase were M2 (R4 = NL 3.1.1.1) and M3 (R1 = FN 263). M2 reached up to 7.8% in the river and 6.2% in the pond waters by Day 2, and was detected until Day 21 in some samples. Maximum mean levels of M3 were 10.2% (river) and 5.8% pond, observed on Day 2. On all other sampling days, the concentrations of Metabolite M3 remained below 1%.
- The amounts of the test item reached maximum values after two days of incubation. At this interval the mean concentrations of [^{14}C]S-Methoprene in the river sediment were 16.6% and 20.8% in the pond sediment. These amounts decreased to 3.3% of applied in the river sediment and to 1.7% in the pond sediment by Day 49. In both sediments M2 (R4 = NL 3.1.1.1) and M3 (R1 = FN 263) were the predominant metabolites, but the levels did not exceed 1.9% of applied in either sediment, and had decreased to levels below 1% by Day 49. One additional metabolite M1 was detected which appeared to be less polar than the parent [^{14}C]S-Methoprene. M1 reached maximum mean levels 1.9% (river, Day 21) and 2.5% (pond, Day 14).

S-Methoprene degrades at a very rapid rate when applied to an aerobic aquatic environment. The primary route of degradation was mineralization (54.9-67.5%). Significant formation of bound residues was observed (36.9- 41.0%). Acidic harsh extraction under reflux followed by organic matter fraction was conducted on the non-extracted residues from the Day 7 only released 1.6% or less of the total radioactivity. Overall, parent S-Methoprene as well as the two major metabolites M2 and M3 showed rapid dissipation from the total system. Neither S-Methoprene nor the metabolites appear to be persistent in the water-sediment system.

S-Methoprene	Test System @ T = 20°C								Converted to T = 12°C	
	Best fit	M(0) %	K ₁ (d ⁻¹)	K ₂ (d ⁻¹)	g	DT ₅₀ (d)*	DT ₉₀ (d)*	x ² error	DT ₅₀ (d)*	DT ₉₀ (d)*
River (water)	SFO	95.9	0.8862	-	-	0.78	2.60	0.554	1.48	4.93
Pond (water)	SFO	99.1	1.2950	-	-	0.54	1.78	0.982	1.02	3.38
River (sediment)	SFO	16.1	0.1855	-	-	3.74	12.4	34.2	7.09	23.5
Pond (sediment)	SFO	20.9	0.1032	-	-	6.72	22.3	7.23	12.8	42.3
River (total system)	SFO	95.7	0.5235	-	-	1.32	4.40	9.98	2.50	8.34
Pond (total system)	DFOP	99.1	0.109	1.223	0.2756	0.87	9.31	3.12	1.65	17.7
M2 (NL 3.1.1.1)										
River (total system)	SFO	0	0.2429	-	-	2.85	9.48	18.5	5.40	18.0
Pond (total system)	SFO	0	0.1329	-	-	5.21	17.32	28.2	9.88	32.9
M3 (FN 263)										
River (total system)	SFO	0	0.5745	-	-	1.21	4.01	21.0	2.29	7.60
Pond (total system)	SFO	0	0.3613	-	-	1.92	6.37	28.6	3.64	12.1

* The values quoted for water and sediment phases refer to dissipation. The total system values refer to degradation

4.1.1.3.6 Biotic degradation in soil

4.1.1.3.6.1 Laboratory soil degradation studies

Aerobic biodegradation

Summary table – aerobic biodegradation in soil- laboratory study											
Method, Guideline, GLP status, Reliability	Test type ¹	Exposure	Test system				Test substance concentration	Incubation period	Degradation DT ₅₀ *	Remarks	Reference
			Soil origin	Soil type	pH	OC %					
Aerobic transformation in soil, OECD 307, GLP, The study was considered acceptable with a	Soil degradation study		Germany	Loamy sand	5.5	1.61	0.52 mg/kg	120 days	1.76 days	See below	Feldmann, S. (2015), Degradation and Metabolism in Four Soils of
			Germany	Sandy Loam	6.0	0.67			1.38 days		
			Germany	Clay	7.1	1.73			1.50 days		

Reliability score of 1.			Germany	Silt Loam	6.29	1.13			1.57 days		[¹⁴ C] S-methoprene Incubated under Aerobic Conditions. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen / Switzerland
¹ Test according to OECD criteria											

* Geomean DT₅₀ for the four soils = 1.55 days

The route and rate of degradation of [¹⁴C]S-methoprene in four soils incubated under aerobic conditions at 20 ± 2 °C in the dark were investigated.

- [¹⁴C]S-methoprene degraded rapidly in all four soils with DT₅₀ values of 0.93, 0.78, 0.79 and 0.83 days in soils I, II, III and IV, respectively. When converted to 12 °C, this gives DT₅₀ values of 1.76, 1.38, 1.50 and 1.57 days in soils I, II, III and IV, respectively.
- Besides the parent compound one metabolite was identified by co-chromatography as Isopropyl (2E,4E)-11-hydroxy-3,7,11-trimethyldodeca-2,4-dienoate (R4) reaching maximum mean amounts of 2.3%, 2.5%, 2.5% and 3.5% of applied on day 1 in soils I to IV, respectively.
- All other radioactive fractions were minor and transient, not exceeding 5.9% of the applied radioactivity at any sampling interval.
- High mineralisation of the radioactive residues to radiolabeled carbon dioxide was observed in all soils reaching maximum levels of 51.1%, 61.5%, 52.4% and 52.4% of the applied radioactivity for soils I to IV, respectively, on day 118.
- The amount of non-extractable radioactivity was also significant, amounting to maximum mean values of 48.6% to 54.1% of the applied radioactivity during the 118-day incubation period. By the end of the study (day 118), the level of bound residues had declined. Organic matter fractionation on day 28 indicated that the majority of the non-extractable radioactivity was bound to the immobile humic acids and humins (26.7 – 41.4% of the applied radioactivity). Lower amounts of radioactivity (6.7 – 12.1% of applied) were detected in the more mobile fulvic acid fraction.

- The main degradation pathway of S-methoprene in soil proceeds via biodegradation beyond Isopropyl (2E,4E)-11-hydroxy-3,7,11-trimethyldodeca-2,4-dienoate (R4) as degradation inter-mediate and several minor and transient fractions. In addition, a significant ¹⁴CO₂ production and formation of bound residues was observed.

4.2 Effects on Environmental Organisms

4.2.3 Terrestrial Compartment

PNECsoil for s-methoprene

Summary table of ecotoxicological studies for soil organisms								
Method, Guideline, GLP status, Reliability	Species	End point	Exposure		Results (mg/kg dwt)		Remarks	Reference
			Design	Duration	NOEC	EC ₅₀ / LC ₅₀		
OECD 222, ISO No.: 11268-2GLP, Reliability: 1	Earthworm (<i>Eisenia fetida</i>)	reproduction (56 d)	Reproduction test	56 d	106	241	Nominal 5% OM	IUCLID section 9.2.2.2
		mortality (28 d)		28 d	213	404		
OECD 232, ISO No.: 11267-2GLP, Reliability: 1	Collembola (<i>Folsomia candida</i>)	reproduction (56 d)	Reproduction test	28 d	47	79.85	Nominal 5% OM	IUCLID section 9.2.5

The value of PNECsoil in the Assessment Report of S-Methoprene was estimated using the equilibrium partitioning method. (PNECsoil = 0.0003 mg/kg wwt). However new terrestrial studies with S-methoprene have been submitted at time of the BIOPREN 50 LFL FLY LARVICIDE CONCENTRATE product dossier submission. The 28 days study result on *Folsomia candida* (Collembola) was a NOEC_{reproduction} of 47 mg/kg soil dry weight a EC_{50, reproduction} of 79.85 mg/kg soil dry weight and a EC_{10, reproduction} of 24.75 mg/kg soil dry weight and for Earthworm the NOEC_{reproduction} was 106 mg/kg soil dry weight (56 day) and the NOEC_{mortality} was 213 mg/kg soil dry weight (28 day). The , EC_{10, reproduction} value of 24.75 mg/kg soil dry weight for collembolans should be used in the PNEC derivation.

The study has been conducted with a high content of organic material in the artificial soil (i.e. 5% peat). This means that the resulting endpoints have to be corrected for differences between the organic matter content of the test soil and that of the standard soil defined for biocides. For the latter, the standard average organic matter content of 3.4% is used to convert the endpoint to a standard soil for biocides resulting in a EC_{10, reproduction} value of 16.83 mg/kg soil dry weight. As additional data on a least two arthropod species are needed to reduce the AF from 100 to 50 for these kind of compounds an AF of 100 has been applied.

PNECsoil = 16.83/100 = PNECsoil is 0.168 mg/kg dwt (= 0.148 mg/kg wwt)

The derived PNEC is fully in line with guidance in Vol IV Part B, but should be considered with care. From the PPP area it is well known that especially larvicides have very specific modes of action. Therefore possibly sensitive species have not been tested. At present, however, there are no tools on how to address this further.

Background fate and ecotox studies

The evaluation of the studies for the active substance provided by the notifier is presented below:

IUCLID 9.2.2.2	Ágh, I	2015	Effects of S-methoprene technical on earthworm (<i>Eisenia fetida</i>) reproduction in a chronic toxicity test, Toxi-Coop Zrt., Study no. 484-222-0675, Unpublished	Y	Babolna Bio Ltd
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Study Comments: IIIA 9.2.2.2	<p>A few minor amendments/additions were made to the summary prepared by the notifier.</p> <p>All environmental parameters were within the ranges defined by the guideline OECD 222.</p> <p>All validity criteria required according to guideline OECD 222 were fulfilled:</p> <ul style="list-style-type: none"> • Mortality was not observed in the untreated control group and 1.3 % mortality was noticed in the vehicle control after the first four weeks. • More than 30 juveniles were produced in each replicate of controls. The mean number of juveniles was 52.0 in the vehicle control and 56.4 in the untreated control. • Coefficient of variance of reproduction in control was 18.3 in the vehicle control and 20.5 in the untreated control. <p>The study has been conducted with a content of 5% organic material in the artificial soil (<i>i.e.</i> peat), the resulting endpoints have to be corrected for differences between the organic matter content of the test soil and that of the standards soil defined for biocides. For the latter, the standard average organic matter content of 3.4% is used to convert the endpoint to a standard soil for biocides.</p> <p>The RMS considers the study reliable without restriction.</p>
Agreed endpoint/s: IIIA 9.2.2.2	<p>Mortality: 28 d NOEC 106 mg/kg soil dwt (nominal; 72 mg/kg soil dwt for a standard soil containing 3.4 % organic matter).</p> <p>Reproduction: 56 d NOEC 213 mg/kg soil dwt (nominal; 145 mg/kg soil dwt for a standard soil containing 3.4 % organic matter).</p>

IUCLID 9.2.5.	Vértesi, A	2015	COLLEMBOLAN REPRODUCTION TEST IN SOIL WITH S-METHOPRENE TECHNICAL, Toxi-Coop Zrt., Study No: 484-232-0676, Unpublished	Y	Bábolna Bio Ltd
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Study Comments: IIIA 9.2.5	<p>A few minor amendments/additions were made to the summary prepared by the notifier.</p> <p>All environmental parameters were within the ranges defined by the guideline OECD 232.</p> <p>All validity criteria required were fulfilled:</p> <ul style="list-style-type: none"> • Mean adult mortality in untreated control and acetone control groups: $\leq 20\%$ (8.75% and 1.25% in test respectively) • Mean number of juveniles per test vessel in untreated control and acetone control groups: ≥ 100 (average 201.8 and 190.4 in test respectively) • Coefficient of variation for the mean number of juveniles in untreated control and acetone control groups: $< 30\%$ (15.46% and 17.19% in test respectively) <p>In a separate study, the EC50 (reproduction) of the reference item boric acid was calculated to be 97.48 mg/kg soil dry weight which is within the range defined by guideline OECD 232. The results of the reference test demonstrate the sensitivity of the test system.</p> <p>The study has been conducted with a high content of organic material in the artificial soil (<i>i.e.</i> 5% peat), the resulting endpoints have to be corrected for differences between the organic matter content of the test soil and that of the standard soil defined for biocides. For the latter, the standard average organic matter content of 3.4% is used to convert the endpoint to a standard soil for biocides.</p> <p>The RMS considers the study reliable without restriction.</p>
Agreed endpoint/s: IIIA 9.2.5	28 d NOEC 47 mg/kg soil dw (nominal; 31.96 mg/kg soil dw for a standard soil containing 3.4% organic matter).

5.1.3 PBT Assessment (following Annex XIII to Regulation (EC) No 1907/2006)

Assessment of persistence

Screening

No soil or aquatic degradation studies were submitted with the original S-methoprene dossier. Rather a range of literature data of variable quality was cited by the Applicant to address the persistence of the active substance. The evaluator for the RMS concluded the following:

"Overall it is considered that there are indications from the literature data supplied that microbial degradation could be expected to be sufficiently rapid such that methoprene would not be persistent in either soil or aquatic systems. This conclusion is also assumed to apply to the S-methoprene enantiomer. Due to deficiencies in the data relied upon, and the fact that the data refers to the racemic mixture of methoprene rather than S-methoprene alone, the P assessment should be regarded as provisional in nature. It could be updated to take account of experimental degradation data on S-methoprene that may be required for product authorisations, which would then allow for a definitive P classification of S-methoprene.

Based on the experimental data submitted here (presented above) it can be concluded that S-methoprene is not persistent in nature. Degradation in soil and aquatic sediment is rapid and does not meet the persistence criteria detailed below. Two major metabolites were detected in the aquatic sediment study. Whole system DT₅₀ values of 9.9 days and 3.6 days for metabolites M2 and M3 respectively indicate that these also do not meet the persistence criteria. No major metabolites were detected in the soil degradation study.

Assessment

P Criteria	Assessment
T1/2 > 60 days in seawater, or	n/a
T1/2 > 40 days in fresh- or estuarine water, or	Parent: No T1/2 = 12.1 days @ 12°C (Whole system value, DFOP kinetics, T1/2 derived from slow phase of degradation curve); M2: No T1/2 = 9.88 days @ 12°C (Whole system value, SFO kinetics); M3: No T1/2 = 3.64 days @ 12°C (Whole system value, SFO

P Criteria	Assessment
	kinetics)
T1/2 > 180 days in seawater sediment, or	n/a
T1/2 > 120 days in freshwater- or estuarine sediment, or	Parent: No T1/2 = 12.1 days @ 12°C (Whole system value, DFOP kinetics, T1/2 derived from slow phase of degradation curve); M2: No T1/2 = 9.88 days @ 12°C (Whole system value, SFO kinetics); M3: No T1/2 = 3.64 days @ 12°C (Whole system value, SFO kinetics)
T1/2 ≤ 120 days in soil.	Parent: No T1/2 = 1.8 days No major metabolites detected

vP Criteria	Assessment
T1/2 > 60 days in sea-, fresh- or estuarine water water, or	Parent: No T1/2 = 12.1 days @ 12°C (Whole system value, DFOP kinetics, T1/2 derived from slow phase of degradation curve); M2: No T1/2 = 9.88 days @ 12°C (Whole system value, SFO kinetics); M3: No

vP Criteria	Assessment
	T1/2 = 3.64 days @ 12°C (Whole system value, SFO kinetics)
T1/2 > 180 days in seawater-, freshwater- or estuarine sediment, or	Parent: No T1/2 = 12.1 days @ 12°C (Whole system value, DFOP kinetics, T1/2 derived from slow phase of degradation curve); M2: No T1/2 = 9.88 days @ 12°C (Whole system value, SFO kinetics); M3: No T1/2 = 3.64 days @ 12°C (Whole system value, SFO kinetics)
T1/2 > 180 days in soil.	Parent: No T1/2 = 1.8 days No major metabolites detected

Conclusion on P / vP properties	Not P and not vP
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