

ECOTOXICITY ELEMENTS
TOXICITY TO TERRESTRIAL ORGANISMS


Soil invertebrates: *Enchytraeus albidus*, *Apporectodea caliginosa*, *Apporectodea longa*,
Folsomia fimetaria, *Hypogastrura assimilis*, *Hypoaspis aculeifer*

PAPER REVIEWED

Holmstrup, M., Krogh, P.H. 2001. Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 3. Sublethal effects on soil invertebrates. *Environmental Toxicology and Chemistry*, 20, 1673-1679.

TEST SUBSTANCE

- (C_{11.6}) LAS (Condea Augusta, Milan, Italy).

 Remarks: The neat material was 16.1 % (w/w) active C_{11.6} LAS in an aqueous sodium salt solution, average molecular weight = 342 g/mol, distribution of the linear alkyl chains: C₁₀ 14 %, C₁₁ 34 %, C₁₂ 31 %, C₁₃ 21 %. Purity was determined by the high-performance liquid chromatographic technique used in the study. All data expressed in mg LAS (active substance) / kg d.w. soil.

METHOD

- Objectives To determine the (sublethal) effects of LAS on 6 soil invertebrates in one soil type, under worst case conditions.
- Method/guideline followed Earthworms *Apporectodea caliginosa* var *tuberculata* and *A. longa*.
 - Reproduction: no internationally accepted guideline available for these species. Important deviations from ISO 11268-2 (Reproduction test with *Eisenia fetida*; ISO 1998): 3 instead of 10 worms per test container; 1 kg soil (dry weight) instead of 500-600 g; 21 days incubation without removal of adults instead of 28 days with adults and another 28 days without adults; water content adjusted after 14 days.
 - ‘Growth’: ‘growth’ was determined as the dry weight after 28 days of exposure of juvenile worms and not as weight increase over time. No internationally accepted guideline available.Enchytraeid *Enchytraeus albidus*: draft ISO/WD 16387: Soil quality – Effects of pollutants on enchytraeidae (*Enchytraeus* sp.) – Determination of effects on reproduction (ISO, 1999a).
Springtails *Folsomia fimetaria* and *Hypogastrura assimilis*: No internationally accepted guideline

available. Test performed according to previously described methods (Wiles and Krogh, 1998). Important deviations from ISO 11267 (ISO 199b): adult springtails instead of juveniles were used; 20 individuals instead of 10 per test container; 21 days exposure instead of 28 days.

Predacious mite *Hypoaspis aculeifer*: no internationally accepted guideline available. Test performed according to a previously described method (Krogh and Axelsen, 1998).

- Test substrate/application
A natural sandy, agricultural soil was used. Defaunation by sieving (2 mm mesh) and heating at 80 °C (time not mentioned). Description of the soil characteristics available in the reviewed manuscript and Holmstrup et al. (2001).
LAS was dissolved in demineralized water and added to the test soil.
- GLP
Likely not.
- Year (study performed)
≤ 2001.
- Species/strain/supplier
A. caliginosa and *A. longa*: collected at an agricultural site near Aarhus, Denmark and maintained in laboratory. Acclimation for 7 days in test soil for the reproduction experiments; laboratory cultures for cocoon production, so that newly hatched juveniles were used in growth tests, after 7 days acclimation in test soil.
E. albidus, *F. fimetaria*, *H. asimilis*, *H. aculeifer*: suppliers or collection sites not mentioned.
- Analytical monitoring
Initial nominal LAS concentrations verified (only in predacious mite test) with HPLC. A limited number of LAS concentrations were determined in a representative series of concentrations (without animals?) (9 and 21 days) for the tests with *H. aculeifer* and the *A. caliginosa* reproduction tests.
- Exposure period
A. caliginosa and *A. longa*: 21 days for the reproduction test; 28 days for the growth test.
E. albidus: 21 days (survival) + 21 days (reproduction).
F. fimetaria, *Hypogastrura asimilis*, *Hypoaspis aculeifer*: 21 days (survival and reproduction).
- Endpoints
Mortality, weight, reproduction.
- Statistical methods
NOEC, LOEC were determined with ANOVA and Dunnet's test. LC10 and LC50 were estimated with

probit analysis, EC10 and EC50 were estimated with model fitting (as described in the manual, SAS 1989) or inhibition concentration approach.

Remarks: A sandy agricultural soil was used instead of an artificial soil and LAS was added as an aqueous sodium salt solution. This may have enhanced bioavailability. The low number of earthworms and the high volume of test soil may have negatively influenced earthworm reproduction (low mating opportunities and hence low cocoon production), although reproduction is low in the species mentioned (e.g. lower than in *Eisenia fetida*). C.V. of reproduction in the control is very high. Therefore, effects on cocoon production were to our opinion not robust enough for consideration in PNEC evaluation.

RESULTS

- Nominal concentrations *A. longa* and *A. caliginosa*, *Hypoaspis aculeifer*, *F. fimetaria*, *Hypogastrura asimilis*: 0, 8, 28, 79, 278, 793 mg LAS / kg d.w.
E. albidus: 0, 20, 40, 80, 198, 397 mg LAS / kg d.w.
Concentrations were derived from tables and graphs in the reviewed paper, since they were not literally mentioned in the text.
- Measured concentrations Good agreement between nominal and measured initial concentrations, with declining concentrations after longer incubation times.
See Table 1.

Table 1: Nominal and measured concentrations of LAS during the experiments.

Species	Time (days)	LAS concentration (mg LAS / kg d.w.) (mean ± standard error)	
		Nominal	determined
<i>Hypoaspis aculeifer</i>	0	0	< 1
	0	8	7 ± 1
	0	28	23 ± 0
	9	28	17 ± 2
	21	28	15 ± 0
	0	79	68 ± 5
	0	278	258 ± 7
	9	278	199 ± 1
	21	278	182 ± 8
	0	793	761 ± 7
<i>A. caliginosa</i> ^a	9	28	22 ± 3
	21	28	13 ± 2
	9	278	245 ± 10
	21	278	190 ± 26

^a In test soil without animals.

- NOEC, LOEC, EC10, EC10, LC50, LC10 See Table 2.

Table 2: NOEC, LOEC, ECx and LCx values (mg LAS / kg d.w.) for the 6 soil invertebrates exposed to LAS.


Species	Parameter	NOEC	LOEC	EC10 or LC10	EC50 or LC50
<i>E. albidus</i>	Adult survival	198	397	194	430 ^a
	Reproduction	20	40	6	41
<i>A. caliginosa</i>	Adult survival	278	793	329	535
	Cocoon production	>793 ^b	>793 ^b	14 ^b	129 ^b
	Juvenile survival	>397	>397	>397	>397
	Juvenile growth	278	397	105 ^c	354 ^c
<i>A. longa</i>	Adult survival	278	793	329	535
	Cocoon production	>793 ^b	>793 ^b	27 ^b	137 ^b
	Juvenile survival	397	793	296	517
<i>F. fimetaria</i>	Juvenile growth	79	278	84 ^c	349 ^c
	Adult survival	>793 ^d	>793 ^d	>793 ^d	>793 ^d
<i>Hypogastrura assimilis</i>	Reproduction	278 ^d	793 ^d	85 ^d	424 ^d
	Reproduction	79	278	99	421
<i>Hypoaspis aculeifer</i>	Adult survival	>793	>793	>793	>793
	reproduction	278	793	82 ^c	236 ^c

^a extrapolated data in the reviewed paper.

^b data not robust enough to our opinion (too low cocoon production) for statistical analysis.

^c Not clear how minimal weight was determined: parameter in the regression calculation or default value (see remarks)?

^d Not clear how the ECx was calculated (no normal dose response).

 Remarks: According to ISO 11268-2 too few replicates were used for the ECx approach (less than 5 in the controls) (ISO 1998). Nominal concentrations were derived from tables and figures, since they were not mentioned in the text per species. It was not clear in the reviewed paper whether minimal juvenile growth (maximal effect) in *Apporectodea spp.* was a parameter or a default value in the ECx regression calculations. The choice for one of both possibilities might influence the resulting ECx.

CONCLUSIONS

According to the authors, this study represents a worst case (sandy test soil, LAS added as an aqueous solution to the soil). A number of results of the original manuscript were omitted in this summary, because they were, to our opinion, not robust enough for the reasons discussed (reproduction in earthworms, ECx calculations for some of the endpoints). ECx calculations were not withdrawn although only 4 instead of 5 replicates for the control were used (ISO 1998).

Overall, the highest sensitivity towards LAS was observed in species in close contact with pore water (EC10 reproduction in *E. albidus* = 6 mg LAS/kg d.w.).

RELIABILITY

Klimisch score (Klimisch *et al.* 1997). See Table 3.

Table 3: Klimisch reliability score for the experiments with the 6 soil invertebrates.

species	endpoint	Klimisch score	reason
<i>E. albidus</i>	Survival and reproduction	1b	Draft ISO/WD 16387 no GLP, LC50 extrapolated.
<i>A. caliginosa</i>	Adult survival	2c (comparable to guideline study with acceptable restrictions)	Comparable to 11268-2
	Cocoon production	3d (unsuitable test system)	Limited number of cocoons, due to species selection and deviations from ISO 11268-2
	Juvenile survival and growth	2c (comparable to guideline study with acceptable restrictions)	Comparable to 11268-2, but only weight measurement, no real growth.
<i>A. longa</i>	Adult survival	2c (comparable to guideline study with acceptable restrictions)	Comparable to 11268-2
	Cocoon production	3d (unsuitable test system)	Limited number of cocoons, due to species selection and deviations from ISO 11268-2.
	Juvenile survival and growth	2c (comparable to guideline study with acceptable restrictions)	Comparable to 11268-2, but only weight measurement, no real growth.
<i>F. fimetaria</i>	Adult survival and reproduction	1b	Comparable to ISO 11267 no GLP.
<i>H. asimilis</i>	reproduction	1b	Comparable to ISO 11267 no GLP.
<i>H. aculeifer</i>	Adult survival and reproduction	2a	No guideline available, no GLP.

General remarks: not all concentrations were literally mentioned in the reviewed paper; ECx calculations were not always fully described.

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