

ECOTOXICITY ELEMENTS
TOXICITY TO TERRESTRIAL ORGANISMS
Soil invertebrates: *Folsomia candida*, *Aporrectodea caliginosa*

PAPER REVIEWED

Holmstrup, M., Krogh, P.H., Løkke, H., de Wolf, W., Marshall, S., Fox, K. 2001. Effect and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 4. The influence of salt speciation, soil type, and sewage sludge on toxicity using the collembolan *Folsomia fimetaria* and the earthworm *Aporrectodea caliginosa* as test organisms. Environmental Toxicology and Chemistry, 20, 1680-1689.

TEST SUBSTANCE

- LAS (Condea Augusta, Milan, Italy).


☞ Remarks: Neat Na-LAS was 16.1 % (w/w) active C_{11.6} LAS in an aqueous solution, average molecular weight = 342 g/mol; Ca-LAS was > 99 % active C_{11.6} LAS (crystalline), average molecular weight = 674 g/mol (Ca is bivalent and therefore, each Ca atom has 2 LAS molecules); Mg-LAS 94 % active C_{11.6} LAS (crystalline), average molecular weight = 658 g/mol (Mg is bivalent and therefore, each Mg atom has 2 LAS molecules). The distribution of the linear alkyl chains was: 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 or soil-sludge mixture, nominal values).

METHOD

- Laboratory National Environmental Research Institute, Department of Terrestrial Ecology, Silkeborg, Denmark.
- Objectives To determine the effects of speciation, soil type, sludge amendment on the toxicity of LAS towards the soil invertebrates *Folsomia fimetaria* and *Aporrectodea caliginosa*
- Method/guideline followed Springtail *Folsomia fimetaria*: No internationally accepted guideline available for this species. Test performed according to a previously described method (Løkke & Van Gestel 1998). Important deviations from ISO 11267:1999(E) (ISO 1999): adult springtails instead of juveniles were used; 20 individuals instead of 10 per test container; 21 days exposure instead of 28 days.
Earthworm *Aporrectodea caliginosa* growth test: no internationally accepted guideline available for growth

in this species. Important deviations from OECD (1984, Earthworm acute toxicity tests): 1 worm in 60 g of natural soil instead of 10 worms in 750 g of artificial soil were used per test container. Juveniles instead of adults were used. Next to survival, 'growth' was determined as the dry weight after 28 days of exposure of juvenile worms and not as weight increase over time.

- Test substrate/application
A sandy, a loamy and a clayey natural soil were used. Defaunation by sieving (2 mm mesh) and heating at 80 °C (time not mentioned). Thorough description of soil characteristics available in Møbjerg and Nielsen (1986). Sludge was obtained from a Danish wastewater treatment plant (Skaevinge, Denmark), receiving water predominantly from non-industrial sources. The sludge was not preconditioned before application. Na-LAS was added as a solution in demineralised water, Ca-LAS and Mg-LAS were added as solutions dissolved in acetone. Controls were set up with acetone. For the earthworm tests, a water control was also included in the Na-LAS experiments. Test soils were equilibrated for 24 h. Sludge was spiked with Na-LAS 1, 7 or 14 days before the start of the experiment at 15 °C.
- GLP
Likely not.
- Year (study performed)
≤ 2001.
- Species/strain/supplier
Suppliers or collection sites not mentioned.
- Analytical monitoring
Nominal LAS concentrations verified with HPLC in representative concentrations for each experiment (without animals?).
- Exposure period
F. fimetaria: 21 days.
A. caliginosa: 28 days.
- Endpoints
F. fimetaria: mortality, reproduction.
A. caliginosa: mortality, growth (determined as weight after 28 days of exposure).
- Statistical methods
NOEC, LOEC with ANOVA and Dunnet's test. LC10 and LC50 were estimated with probit analysis, EC10 and EC50 were estimated with model fitting or inhibition concentration approach.

 Remarks: LAS speciation was not measured during the experiments.

RESULTS

- Nominal concentrations See Table 1.

Table 1: Nominal concentrations of LAS (mg / kg d.w.) during the experiments.

Species	Salt speciation test			Soil type test	Sludge test
	Na-LAS	Ca-LAS	Mg-LAS	Na-LAS	Na-LAS
<i>F. fimetaria</i>	0	0	0	0	0
	99	118	113	8	64
	198	235	227	28	128
	397	470	453	79	256
	595	705	679	278	384
	793	940	905	793	512
<i>A. caliginosa</i> ^a	0	0	0	0	0
	40	45	45	40	26
	80	94	92	79	51
	160	188	181	159	103
	272	327	317	278	179
	397	469	453	397	256

^a Concentrations derived from figures and tables, since they were not literally mentioned in the reviewed manuscript.

- Measured concentrations Good agreement between nominal and measured initial concentrations (discrepancies of $\pm 15\%$), with declining concentrations after longer incubation times (20-40 % after 21 days).
- NOEC, LOEC, EC10, EC10, LC50, LC10 See Tables 2-4.

Table 2: NOEC, LOEC, ECx and LCx values (mg LAS / kg d.w.) for *F. fimetaria* (21 days) and *A. caliginosa* (28 days) exposed to LAS: effect of salt speciation.

Species	Endpoint	LAS type	NOEC	LOEC	EC10 or LC10	EC50 or LC50
<i>F. fimetaria</i>	Adult survival	Na-LAS	>793	>793	>793	>793
		Ca-LAS	>940	>940	>940	>940
		Mg-LAS	>905	>905	>905	>905
	Reproduction	Na-LAS	595	793	87 ^a	434 ^a
		Ca-LAS	705	940	106	528
		Mg-LAS	227	453	97	487
<i>A. caliginosa</i>	Juvenile survival	Na-LAS	N.A.	N.A.	148	344
		Ca-LAS	N.A.	N.A.	282	630
		Mg-LAS	N.A.	N.A.	197	480
	Growth	Na-LAS	397	>397	90 ^a	381 ^a
		Ca-LAS	94	188	61	175
		Mg-LAS	181	317	91 ^a	251 ^a

^a ECx also calculated according to Vanewijk and Hoekstra (1993) (see Table 5) because hormesis was not taken into account in the reviewed study.
N.A. Data insufficient to calculate NOEC or LOEC.

Table 3: NOEC, LOEC, ECx and LCx values (mg LAS / kg d.w.) for *F. fimetaria* (21 days) and *A. caliginosa* (28 days) exposed to LAS: effect of soil type.

Species	Endpoint	Soil type	NOEC	LOEC	EC10 or LC10	EC50 or LC50
<i>F. fimetaria</i>	Adult survival	Sand	≥793	>793	>793	>793
		Loam	≥793	≥793	2	143
		Clay	79	278	77	345
	Reproduction	Sand	278	793	85	424
		Loam	350	793	4	34
		Clay	79	278	93 ^a	208 ^a
<i>A. caliginosa</i>	Juvenile survival	Sand	≥397	≥397	≥397	≥397
		Loam	≥397	≥397	≥397	≥397
		Clay	≥397	≥397	≥397	≥397
	Growth	Sand	278	397	105 ^a	354 ^a
		Loam	40	79	44 ^a	180 ^a
		Clay	79	159	59 ^a	169 ^a

^a ECx also calculated according to Vanewijk and Hoekstra (1993) (see Table 6) because hormesis was not taken into account in the reviewed study.

Table 4: NOEC, LOEC, ECx and LCx values (mg LAS / kg d.w.) for *F. fimetaria* (21 days) and *A. caliginosa* (28 days) exposed to Na-LAS: effect of sludge amendment.

Species	Endpoint	Incubation time (d)	NOEC	LOEC	EC10 or LC10	EC50 or LC50
<i>F. fimetaria</i>	Adult survival	1	≥512	≥512	440	>512
		7	128	256	75	956
		14	64	128	32	209
	Reproduction	1	128	256	112	324
		7	128	256	94 ^a	235 ^a
		14	64	128	73	108
<i>A. caliginosa</i>	Juvenile survival	1	≥256	>256	>256	>256
		7	≥256	>256	>256	>256
		14	≥256	>256	225	493
	Growth	1	103	179	38	240
		7	26	51	14	139
		14	26	51	19	100

^a ECx also calculated according to Vanewijk and Hoekstra (1993) (see Table 7) because hormesis was not taken into account in the reviewed study.

Remarks: According to ISO 11268-2 too few replicates were used for the ECx approach (less than 5 in the controls) (ISO 1998). Since LAS speciation was not measured during the tests and the test soils were agricultural (limed) soils, it is possible – as the authors stated – that Ca-, Na- and Mg-LAS were all actually present in the test soils as Ca-LAS (after Ca substituted Na and Mg).

CONCLUSIONS

The authors suggest that neither chemical characterization (salt type) of LAS nor soil type or application of sludge to test soils have any significant influence on the toxicity towards *F.*

fiimetaria and *A. caliginosa*. However, it is possible that Na-, Ca- and Mg-LAS were all three present during the tests as Ca-LAS and therefore the speciation results must be interpreted with caution (no extrapolation to sandy and/or unlimed soils is possible). Overall, the lowest EC10 was found for survival of *F. fiimetaria* exposed to Na-LAS in sandy loam (2 mg LAS / kg d.w.).

RELIABILITY

Klimisch score (Klimisch *et al.* 1997, PAGES P&G?) See Table 3.

Table 3: Klimisch reliability score for the experiments with *F. fiimetaria* and *A. caliginosa*.

species	Klimisch score	Reason
<i>F. fiimetaria</i>	1b	Comparable to ISO 11267 (ISO 1999)
<i>A. caliginosa</i>	2a	Comparable to OECD guideline no. 207 (OECD 1984)

REFERENCES

- ISO. 1999. Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. ISO 11267, Geneva, Switzerland.
- Klimisch, H.-J., Andreae, M., Tillmann, U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, 25, 1-5.
- Møbjerg, J., Nielsen, J. 1986. The constituent composition of soils from Danish state agricultural research stations. *Tidsskrift Planteavl S*, 1870, 1-37.
- OECD. 1984. Earthworm, acute toxicity test. OECD guideline for testing of chemicals no. 207.
- Vanewijk, P.H., Hoekstra, J.A. 1993. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. *Ecotoxicology and Environmental Safety*, 25, 25-32.

ECx calculations performed by the reviewer (Dr. B. Versonnen, Ghent University)

Tables 5-7 represent the results of our ECx calculations based on mean values of the experiments and not on the raw data. As the data presented are based on the means given in the reviewed paper and not on the raw data, they must be interpreted with caution (no statistical significance for the parameters in the model of Vanewijk and Hoekstra, 1996). As a comparison with the data in the original paper (no hormesis), we fitted the hormesis model to the data, as recommended (but not implemented) by the authors of the reviewed paper.

Table 5: Calculated ECx values and confidence intervals, based on means (mg LAS / kg d.w.) for *F. fiimetaria* (21 days) and *A. caliginosa* (28 days) exposed to LAS: effect of salt speciation, performed according to Vanewijk and Hoekstra (1993).

species	Endpoint	Salt	EC10	EC50	Hormesis
<i>F. fiimetaria</i>	Reproduction	Na-LAS	258	578	Yes

<i>A. caliginosa</i>	Growth	Na-LAS	(45-1495) 151	(114-2932) 628	Yes
		Mg-LAS	(20-11343) 101	(1-3.5E5) 230	Yes
			(14-717)	(104-508)	

Table 6: Calculated ECx values and confidence intervals, based on means (mg LAS / kg d.w.) for *F. fimetaria* (21 days) and *A. caliginosa* (28 days) exposed to LAS: effect of soil type, performed according to Vanewijk and Hoekstra (1996).

species	Endpoint	Soil type	EC10	EC50	Hormesis
<i>F. fimetaria</i>	Reproduction	Clay	432 (402-463)	525 (495-557)	Yes
<i>A. caliginosa</i>	Growth	Sand	145 (88-241)	458 (247-847)	Yes
		Loam	N.A.	N.A.	Yes
		Clay	71 (18-276)	167 (84-334)	Yes

N.A. = data insufficient for ECx calculations.

Table 7: Calculated ECx values and confidence intervals, based on means (mg LAS / kg d.w.) for *F. fimetaria* (21 days) exposed to LAS: effect of sludge incubation time, performed according to Vanewijk and Hoekstra (1996).

species	Endpoint	Incubation time	EC10	EC50	Hormesis
<i>F. fimetaria</i>	Reproduction	7	171 (109-269)	349 (274-444)	Yes