ECOTOXICITY ELEMENTS
TOXICITY TO TERRESTRIAL ORGANISMS
Soil microorganisms, short-term laboratory study

PAPER REVIEWED

Elsgaard, L., Petersen, S.O., Debosz, K. 2001. Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 1. Short-term effects on soil microbiology. Environmental Toxicology and Chemistry, 20, 1656-1663.

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: C_{10} 14 %, C_{11} 34 %, C_{12} 31 %, C_{13} 21 %. Purity was determined by high-performance liquid chromatography described in Holmstrup et al. (2001). All data expressed in mg aqueous LAS (active substance) / kg d.w. soil.

METHOD

- Laboratory: Danish Institute of Agricultural Sciences, Department of Crop Physiology and Soil Science, Tjele, Denmark.

- Objectives: To determine the short-term effects of aqueous LAS (0-793 mg/kg d.w.) added to a natural soil, on 10 microbial soil parameters related to C and N transformation, enzymatic activity, anaerobic activity, microbial populations and a broad indicator of microbial biomass.

- Method/guideline followed: No guidelines available. All methods fully described in the reviewed paper.

- Test substrate/application: Soil: an agricultural coarse sandy soil in Lundgaard, Denmark. Description of the soil characteristics in the reviewed paper and in Holmstrup et al. (2001). Sludge had never been applied before to the soil and pesticides had not been sprayed the previous 2 years. After sampling, the soil was stored at 2 °C (13.5 % water content) for 2 months. The soil was acclimated at 15 °C for 4 weeks and sieved (4 mm) before the experiments.
  For each LAS concentration, soil was amended with the appropriate LAS solution (freshly prepared from the stock solution). All soils were carefully mixed and incubated at 15 °C. Assays of potential ammonia oxidation (PAO), potential dehydrogenase activity

(PDA), β-glucosidase activity, cellulolytic microorganisms and phospholipids fatty acids (PLFA) were done with subsamples from the same batches of LAS-amended soil. Assays of ethylene (C_2H_4) degradation, iron reduction and basal soil respiration (BR) were done with separate batches of LAS-amended soil. For each of these assays, further details on LAS amendment to the soil, incubation and number of replicates are fully described in the reviewed paper.

- GLP: Likely not.

- Year (study performed): 1997

- Analytical monitoring: For LAS analyses, duplicate soil samples of each batch (except batch prepared for BR measurements) were collected at the start of the incubation period and frozen (all LAS levels). LAS was determined with HPLC as described in Holmstrup et al. (2001).

- Exposure period: Assays of PAO, PDA, β-glucosidase activity and cellulolytic microorganisms were done after incubation and LAS exposure for 7 d, PLFA analysis after incubation and LAS exposure for 11 d.
  The C_2H_4 degradation rate (h^{-1}) was averaged over a time period of 7 h, starting 3 h after LAS amendment to the soil. The iron reduction was tested after incubation and LAS exposure for 5 d. BR was measured after incubation and LAS exposure for 1, 2, 4, 5 and 9 d.

- Endpoints: C_2H_4 degradation, PAO, PDA, β-glucosidase activity, iron reduction, cellulolytic microorganisms (cellulose-utilizing bacteria, fungi and actinomycetes), BR and PLFA

- Statistical methods: EC_{10} and EC_{50} values were calculated by a linear-interpolation analysis (IC_p), which was based on bootstrapping. The NOECs and LOECs were determined by Dunnett's test using a SAS analysis-of-variance procedure.

Remarks: Due to the experimental design, the results from the C_2H_4 degradation assay were treated as pseudoreplicates in terms of LAS effects. All other microbial tests, however, were carried out as true replicates.

RESULTS

- Nominal concentrations: 0, 8, 22, 62, 174 and 488 mg LAS / kg d.w. soil, except for BR (0, 0.8, 8, 79 and 793 mg LAS / kg d.w. soil).

- Measured concentrations — On average, 84 to 95 % of the nominal concentrations were initially recovered by the chemical analysis. Nominal levels were used for the calculation of effect concentrations

- NOEC, LOEC, EC_{10}, EC_{50} — See Table 1. Results for each endpoint are briefly described in Table 2.

Table 1: EC_{10}, EC_{50}, NOEC and LOEC values (mg LAS / kg d.w.) for LAS toward microbial parameters in agricultural soil.

Microbial parameter	Incubation	EC_{10}		EC_{50}		NOEC	LOEC
		mean	95% CL	mean	95% CL		
C_2H_4 degradation	10 h	9	N.A.	24	N.A.	N.A.	N.A.
PAO	7 d	< 8	(2-8)	40	(24-76)	0	8
PDA	7 d	22	(6-47)	128	(99-154)	22	62
β-glucosidase activity	7 d	47	(24-63)	> 488	N.A.	174	488
Iron reduction	5 d	< 8	(2-6)	17	(15-18)	0	8
Cellulolytic bacteria	7 d	11	(0-12)	24	(15-40)	8	22
fungi	7 d	< 8	(0-20)	32	(11-76)	8	22
actinomycetes	7 d	8	(0-19)	80	(0-206)	8	22
BR	1-9 d	> 793	N.A.	> 793	N.A.	> 793	> 793
PLFA	11 d	> 488	N.A.	> 488	N.A.	> 488	> 488

95% CL = 95 % confidence limits
N.A. = Not available

Table 2: Results of the different microbial parameters tested in the lab after LAS amendment to soil.

Parameter	Most important results
C_2H_4 degradation	LAS had an inhibitory effect on C_2H_4 degradation, which almost ceased at the highest LAS levels. C_2H_4 degradation showed a similar response to LAS after 10 h and 6 d of LAS exposure, showing that the inhibitory effect occurred rapidly and no recovery occurred within 6 d.
PAO	The inhibitory effect of LAS on PAO was significant even at 8 mg LAS / kg d.w. and was complete at 488 mg LAS / kg d.w.
PDA	PDA was progressively inhibited in soil at increasing LAS concentrations. However, this parameter was less sensitive to LAS than the other microbial parameters (cf. higher EC_{10} and EC_{50}), likely due to the fact that PDA comprises the response of many groups of microorganisms.
β-glucosidase activity	LAS inhibited β-glucosidase activity by only 25% at the highest concentration (488 mg LAS / kg d.w.), which is in accordance with the theory of LAS toxicity (since it is assumed that LAS interacts with cell membranes, higher concentrations of LAS would be expected for inhibition of soil enzymatic processes than for inhibition of soil processes depending on the integrity of microbial cell membranes).
Iron reduction	Bacterial iron reduction was the process most sensitive to LAS, being completely inhibited at 62 mg LAS / kg d.w. This increased toxicity may be due to increased bioavailability since LAS exposure

	during this test was done in a soil-water slurry. Furthermore, EC_{10} and EC_{50} values were lower than found in previous studies of LAS toxicity towards iron reduction, which, apart from the different soil type, can be ascribed to the different soil incubation temperature (15 °C instead of 20 °C).
Cellulolytic microorganisms	The three groups of cellulolytic microorganisms showed a similar and sensitive response to LAS, which probably reflects that microbial cellulose degradation is a complicated process performed by specialized organisms. This study is the first to report effects of LAS on cellulolytic microorganisms.
BR	LAS caused a slight increase in CO_2 production at the highest LAS contents. A partial or complete compensation of inhibitory LAS effects may occur in terms of CO_2 production, even though a decrease in biomass or important changes in microbial community structure and stability have taken place.
PLFA	No effect of LAS on the total PLFA concentration was detected. This lack of response needs to be further investigated, since it is not clear whether these results represent a true indication of unchanged microbial biomass or if some methodological problems were involved.

Remarks: For three soil parameters (PAO, iron reduction and cellulolytic fungi), IC_p estimates for EC_{10} were found that were less than the lowest LAS concentration tested (8 mg LAS / kg d.w.). However, data analysis by arithmetic interpolation pointed toward EC_{10} values of approximately 5 mg LAS / kg d.w. for the three parameters. Therefore, the authors of the reviewed manuscript suggested that these EC_{10} estimates can tentatively, but not unreasonably, be used for the three soil parameters under the present experimental conditions.

CONCLUSIONS

Whereas LAS inhibited specific compartments of the soil microbial community, this could not be deduced from the respiratory activity or the total PLFA content. Values for EC_{10} in the range of approximately 5 to 22 mg LAS / kg d.w. were found for the 7 most LAS-sensitive soil functions and populations. These effect concentrations were slightly higher than the PNEC for plants and soil fauna (~ 5 mg LAS / kg d.w.) that was derived in Jensen et al. (2001).

The documented short-term effects of LAS could be of general importance in agricultural soil. However, LAS contamination in agricultural soils generally occurs through the application of sewage sludge instead of through direct addition of aqueous LAS sodium salt to the soil. The latter represents a situation with high bioavailability and, therefore, high toxicity of LAS.

RELIABILITY

Klimisch score	2a (acceptable, well-documented publication which meets basic scientific principles): no GLP, EC_x

calculation not fully detailed

REFERENCES

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.

Jensen, J., Løkke, H., Holmstrup, M., Krogh, P.H., Elsgaard, L. 2001. Effects and risk assessment of linear alkylbenzene sulfonates in agricultural soil. 5. Probabilistic risk assessment of linear alkylbenzene sulfonates in sludge-amended soils. Environmental Toxicology and Chemistry, 20, 1690-1697.