

ECOTOXICITY ELEMENTS
TOXICITY TO SEDIMENT ORGANISMS
Freshwater benthic organisms, laboratory ecotoxicity study

PAPER REVIEWED

Comber, S.D.W., Conrad, A.U., Hurst, K., Höss, S., Webb, S., Marshall, S. Body burdens and chronic toxicity of sediment-associated linear alkylbenzene sulphonates (LAS) to freshwater benthic organisms. Unpublished manuscript.

TEST SUBSTANCE

- Non-labelled test chemical: (C_{11.4}) LAS (Nansa HS 80 S, provided by Unilever, molecular weight not mentioned).
- Radio-labelled tracer: 3-dodecylbenzene sulphonate (3-DOBS) (95% purity, specific activity of 72.6 µCi/mg, molecular weight = 340 g/mol) with the ¹⁴C labelled carbon atom included in the benzene ring.

 Remarks: LAS concentrations were calculated by multiplying measured values of ¹⁴C-DOBS by the ratio of non-labelled to labelled LAS spiked into the sediment. All data expressed in mg LAS (active substance) / kg d.w. sediment and for body burdens in mmol LAS / kg d.w.

METHOD

- Laboratory *Lumbriculus variegatus* test: WRc-NSF, Buckinghamshire, U.K.
Caenorhabditis elegans test: ECOSSA, München, Germany.
- Objectives To assess the toxicity of LAS to the freshwater benthic organisms *L. variegatus* and *C. elegans* during exposure to LAS-spiked sediment and to measure the body burdens of LAS in *L. variegatus*.
- Method/guideline followed Methods insufficiently described in the reviewed paper (cf. remarks in the paper).
 - *L. variegatus* test: 10 adult *Lumbriculus* were added to 20 g of prepared sediment (wet weight) with 30 ml of overlying groundwater. Each test concentration was replicated 6 times. After 28 days, all live *Lumbriculus* were recovered, counted, wet weighed and dry weighed after 48 h of drying.
 - *C. elegans* test: a bacterial suspension (*E. coli*) was added as food for the nematodes before start of the assay. 10 juvenile worms were then added to LAS-

and bacterial-spiked sediment. Each test concentration was replicated 5 times. After 3 days, nematodes were killed and extracted from the sediment. Body length (growth) and number of eggs inside the body (egg production) were determined under a microscope. Fertility was determined as % of gravid worms (≥ 1 egg inside the body).

- Test substrate/application
 - *L. variegatus* test: a natural uncontaminated sediment (the water content of the sediment and the nature of the contaminants measured in the sediment not mentioned) containing 44 % sand, 48 % silt and 8 % clay with a total organic carbon content of 1.7 % was spiked with aliquots of non-labelled LAS (in dichloromethane) and radio-labelled material (in methanol). Samples were thoroughly mixed for 1 h. It is not clear how the nominal concentrations were obtained (e.g., 600 mg/kg using 0.44 ml of a 100 mg/ml (= 44 mg LAS) in 10 g sediment dry weight + 150 g sediment wet weight (= total of 83.5 g sediment d.w. when water content of sediment = 51%). This results in 44 mg LAS / 83.5 g sediment d.w. = 527 mg / kg d.w and not 600 mg / kg d.w.).
 - *C. elegans* test: an artificial sediment (properties not mentioned) was mixed with unlabelled LAS stock solution (in distilled water). The spiked sediment was incubated for 24 h before the start of the test (temperature not mentioned). It is not clear how the nominal concentrations were obtained (not detailed).

- GLP
 - Likely not.

- Year (study performed)
 - 2001?

- Species/strain/supplier
 - L. variegates*, *C. elegans*: supplier or collection site not mentioned.

- Analytical monitoring
 - *L. variegatus* test: Total ^{14}C was determined in sediment and in overlying water at day 0 and day 28 and in worms after 28 days by LSC. All unlabelled concentrations were calculated using the specific activity of 3-DOBS. To measure degradation of LAS, sediment and overlying water samples were taken for HPLC analysis.
 - *C. elegans* test: LAS sediment concentrations were confirmed by HPLC.

- Exposure period
 - L. variegatus*: 28 days
 - C. elegans*: 3 days

- Endpoints
 - *L. variegates* test: Survival, reproduction, wet/dry weight and body burdens (mmol LAS / kg d.w.). Survival and reproduction are treated as a single endpoint (i.e., total number of organisms at test termination), since *L. variegatus* generally reproduces in tests > 10 days and it is impossible to differentiate between young and adult organisms.
 - *C. elegans* test: growth, egg production and fertility.
- Statistical methods
 - Not mentioned.

📄 Remarks: cf. paper

RESULTS

- Nominal concentrations
 - *L. variegatus* test: 0, 53, 80, 107, 160, 293 and 600 mg LAS / kg d.w. sediment.
 - *C. elegans* test: control, 10, 50, 75, 100, 200, 300, 400, 500, 750 and 1000 mg LAS / kg d.w. sediment.
- Measured concentrations
 - *L. variegatus* test:
 - Sediment: On average, measured concentrations were initially 106 % of the nominal concentrations. The average proportion of LAS still present in the sediment after 28 days was 30 % ± 7 % (95 % confidence interval) of the initial measured concentration. These data provided a calculated half-life of 25 days.
 - Overlying water: On day 0, the average LAS concentration in the overlying water was 0.26 % of the sediment concentration. Dissolved concentrations after 28 days were below the limit of detection (value of limit of detection not mentioned).

All measured concentrations are given in tables in the reviewed paper. Exposure concentrations were expressed as nominal, initial measured concentration and the mean of day 0 and day 28 values.
 - *C. elegans* test: measured concentrations were 73 to 80 % of nominal values after 3 days. Initial concentrations were not measured and were assumed to be 100 % of nominal concentrations. Average exposure was therefore assumed to be 87 to 90 % of nominal.
- NOEC, LOEC, LC₅₀
 - L. variegatus* test: see Table 1.
 - C. elegans* test: see Table 2.

Table 1: NOEC and LOEC values for survival/reproduction and growth of *L. variegatus* and LC₅₀ for *L. variegatus* in sediment during LAS exposure for 28 days (mg LAS / kg d.w.).

	NOEC	LOEC	LC ₅₀
Based on nominal values	107	160	150 (131-170)
Based on measured day 0 values	136	170	164 (150-189)
Based on mean of measured day 0 & day 28 values	81	110	105 (92-118)

For LC₅₀, the 95% confidence limits are given in parentheses.

Table 2: NOEC, LOEC and EC₁₀ or EC₃₀ values for *C. elegans* in sediment during LAS exposure for 3 days (mg LAS / kg d.w.; nominal concentrations).

Test parameter	NOEC	LOEC	EC ₁₀	EC ₃₀
Growth	200	300	275	
Fertility	200	300		258
Egg production	100	200		125

- Body burdens in *L. variegatus* Body burdens in *L. variegatus* ranged from 3226 - 9767 mg LAS / kg d.w. (or 9.49 - 28.7 mmol LAS / kg d.w.) for sediment concentrations from 83 - 160 mg LAS / kg d.w. sediment. The mean biota-sediment accumulation factor (BSAF) was calculated to be 83. When normalised for worm lipid content (1.6 %) and sediment organic carbon content (1.7 %), the BSAF was 88.

Remarks: cf. paper. It is not clear how the LAS body burdens in *L. variegatus* were determined. Total ¹⁴C was determined by LSC but how was this converted to total LAS concentration?

CONCLUSIONS

The study indicates that LAS has a half-life in aerobic sediments of approximately 25 days. The results of the two bioassays demonstrate the relatively low toxicity (LOECs >100 mg/kg dw) and bioaccumulation (BSAF <100) of LAS to the studied test organisms.

Endpoints from these studies in conjunction with other sediment effects data from other species are suitable to derive a PNEC (predicted-no-effect-concentration) for LAS in the sediment compartment (EU 2002). A deterministic PNEC can be derived by application of an assessment factor of 10. This factor is used for PNEC determination when long term NOECs for at least three species representing three trophic levels are available. A chronic ecotoxicological study on *Chironomus riparius* exposed to sediment associated LAS generated a NOEC value of 319 mg/kg d.w. (Pittinger *et al.* 1989). Integrating the two NOEC values generated in the present study, the lowest value was found to be 81 mg/kg d.w. (*L. variegatus*). This yields a sediment PNEC for LAS of 8.1 mg/kg dw.

RELIABILITY

Klimisch score 3b (documentation insufficient for assessment): no

GLP, methods insufficiently described, statistical methods not mentioned

REFERENCES

- EU 2002. Revised technical guidance document for risk assessment of new and existing chemicals and biocidal products. European chemicals bureau, European Commission.
- Pittinger, C.A., Woltering, D.M., Masters, J.A. 1989. Bioavailability of sediment-sorbed and aqueous surfactants to *Chironomus riparius* (midge). *Environmental Toxicology & Chemistry*, 8, 1023 - 1033.