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Biodegradation of DEHP, DBP, and DINP: Poorly Water Soluble and Widely Used Phthalate Plasticizers

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Environmental concentrations of man made chemicals are the net result of emissions during production, use, deposition and the subsequent degradation processes. The later can be distinguished into biotic and abiotic degradation. Very often, monitoring data are lacking, and a precise determination of the biodegradation rate is crucial for correct forcasts of the fate of substances in risk assessment. In case of difficult substances, i.e. substances which are poorly water soluble, an appropriate test design is of utmost importance. The following report deals with the biodegradation of 3 phthalate esters, a group of poorly water soluble substances used mainly as plasticisers. Di-ethylhexyl-phthalate (DEHP, CAS - No. 117-81-7) and di-iso nonylphthalate (DINP, CAS - No. 28553-12-0) are the main platicisers for PVC-P applications and plastisols, whereas di-n-butyl phthalate (DBP, CAS-No. 84-74-2) has its main application areas in adhesives, lacquers and printing inks.

MATERIALS AND METHODS

All platicisers employed are poorly water soluble substances. This is especially true for DEPH and DINP, which show a true water volubility in the lower µg/l - range. DBP is slighthly more soluble, values reaching the lower mg/l - range (ECPI,1995). Up to now, there is only one internationally accepted test system, which is specially designed for the test on biodegradability of such substances, the modified Sturm test (OECD, 1993; EEC, 1992). In this test system, the test substance is incubated in a mineral medium and inoculated with a minor amount of sewage sludge. No further carbon source is offered to the organisms during the test. The system is sealed airtight and flushed with CO_2 free air. The amount of carbon dioxide generated during the 28 days exposure is then determined. The tests have been performed as outlined

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in both the guidelines. Special care has been taken to introduce the test substance into the Sturm test vessels. The phthalates have been dosed into precleaned Eppendorf vials at an amount corresponding to 15 mg organic carbon by direct weighing. One day before the start of the test, the vessels are filled with the mineral medium and the prewashed inoculum, sealed airtight and flushed with CO_2 free air. The Eppendorf vials are placed within the Sturm test bottles by opening a small stop cock, The whole system is subsequently closed airtight again and flushed with CO_2 free air.

All CO_2 evolved during the test is sampled in traps filled with 0.05 N NaOH. From these traps, samples are taken at regular intervals, the first one already after 30 minutes. This is to compensate for traces of CO_2, which may enter the system during the introduction of the test substances.

As biological inoculum a sludge sample from a nearby sewage treatment plant, treating household sewage only, was employed. After collecting the sludge from the plant, it was washed thoroughly with an artificial mineral medium as described in the guidelines. The sludge was thoroughly mixed with the final washing solution and an appropriate amount was then used as inoculum. The amount was such to give a final concentration of 29 mg/l suspended solids, which corresponds to roughly 60 x 10^4 cfu / ml (colony forming units).

C O_2 evolution has been measured with a cabon analyzer TOC 500 (Shimadzu), by injection of 10-20 µl of a sample of the carbon trap content. The evolved carbon dioxide was put into relation to the carbon content of the sample introduced, and the amount of degradation was calculated over the sampling time. The test stopped after 28 days. The total content of the Sturm test vessel is then acidified to drive out remaining CO_2 traces from the vessel into the NaOH trap.

Each phthalate was run separately, but in two parallels. Sodium benzoate served as positive control to cheque on the sufficient performance of the inoculum. A second control without any organic carbon was set up to compensate for any CO_2 release during sample introduction and the self aspiration of the inoculum. All phthalates are commercial products of Hüls AG.

RESULTS AND DISCUSSION

The biodegradation of various phthalate esters have been investigated in a test system, which cheques on the ready biodegradability of substances. The modified Sturm test is especially designed for poorly water soluble substances. Table 1 summarizes the result of the bidegradation rates for

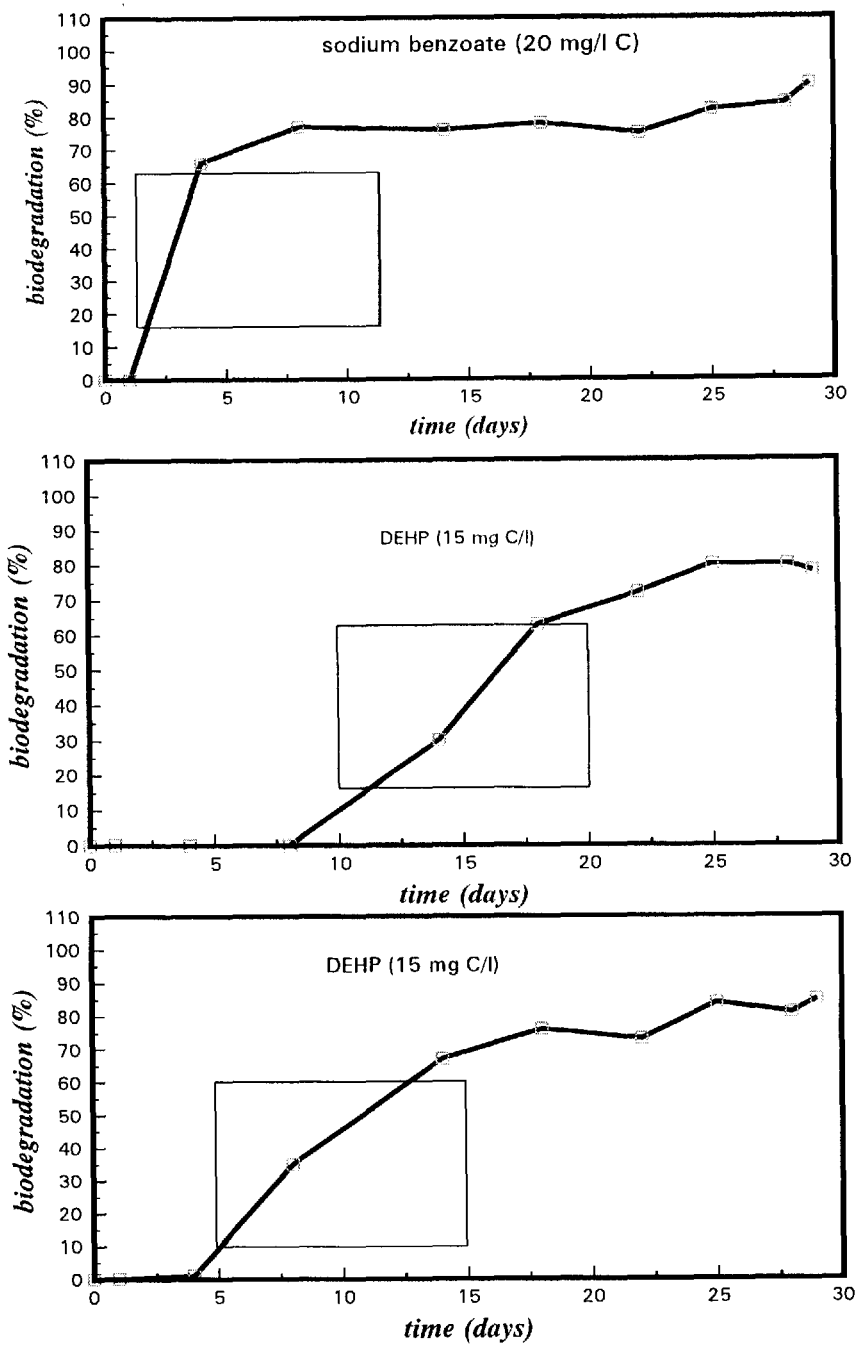

Figure 1. Modified Sturm test. Biodegradation of DEHP and the positive control, sodium benzoate; the inserted box indicates the 10 day window.

DEHP and the corresponding sodium benzoate control. It can be seen that both the DEHP parallels drift apart during the first 8 days of the test. This is certainly be due to an altered biovailability, which may be different in the two test sets. Nevertheless, both sets reach a final degradation rate of 80 and 81% respectively.

Table 1. Modified Sturm test. Biodegration rates for DEHP and the corresponding positve control, sodium benzoate over 28 days.

	Biodegradation rate (%)			
time (days)	Sodium benzoate	DEHP Set 1	DEHP Set 2	DEHP mean
0.5 h	o	0	0	0
1	0	0	0	0
4	66	0	1	1
8	77	0	35	18
14	76	30	67	49
18	78	63	76	70
22	75	72	73	73
25	82	80	84	82
28	84	80	81	81
29*	90	78	85	82

*measurement after final acidification

Figure 1 illustrates the time dependence of the degration process. The box within the figures visualizes the 10 day window. Starting with the onset of a 10% biodegradation rate, it is within this time period that a substance has to be biodegraded by more than 60 % in order to be rendered readily biodegradable. The control demonstrates the good performance of the sludge, showing a rapid onset of the degradation process, a fast increase, and a high final degradation rate of 84 %.

Table 2. Modified Sturm test. Biodegradation rate of DBP and the corresponding positive control, sodium benzoate over 28 days. The standard deviation in based on 4 separate parallels.

	Biodegradation rate (%)		
time (days)	sodium benzonate	DBP*	standard deviation
0.5 h	o	0	
1	13	0	
4	69	42	6
8	76	56	3
13	80	69	2
18	83	73	3
22	87	76	3
28	88	81	4
29*	86	81	4

*mean of separate test vessels

The corresponding figures for DEHP give a similiar impression as for the control. The margin of 60 % degration is reached within the 10 day window period, and with a total biodegration rate of 80 %, we can consider DEHP as readily and completely biodegradable.

The corresponding numbers for DBP are summarized in Table 2. The biodegration pattern of DBP shows a greater coherence within the 4 subsets, which have been tested. The final value ranged from 79 % to 85 %. The graphical outline is summarized in Fig. 2. Quite a rapid onset of the CO_2 evolution can be observed, and, starting with the 10 % value, the minimum biodegration of 60 % is easily accomplished within the next 10 subsequent days. Clearly, the 10 day window has been reached, and DBP can be considered as readily biodegrable.

In case of DINP, the biodegradation rates determined have been compiled

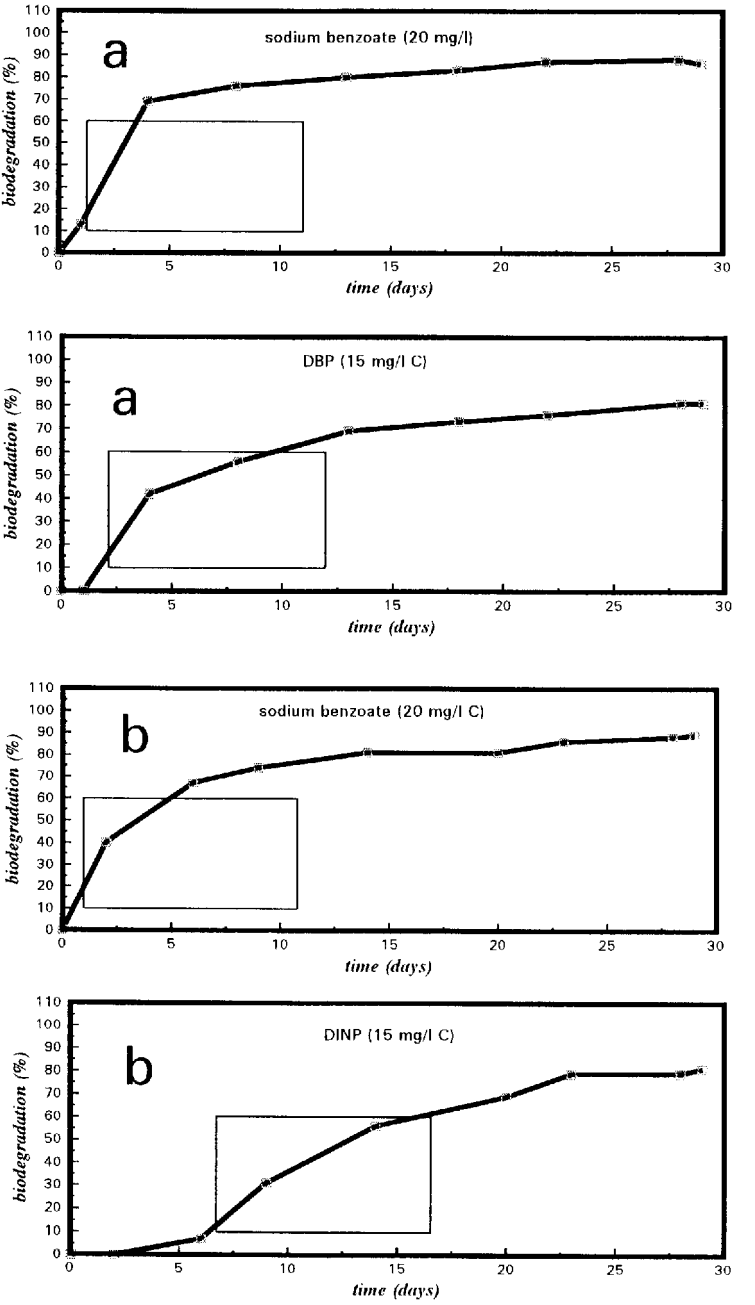

Figure 2a and 2b. Modified Sturm test. Biodegradation of DBP and DINP and their corresponding positive controls, sodium benzoate; the inserted box indicates the 10 day window.

Table 3. Modified Sturm test. Biodegradation rate of DINP and the corresponding positive control, sodium benzoate over 28 days

	Biodegradation rate (%)			
time (days)	sodium benzoate	DINP set 1	DINP set 2	DINP mean
0.5 h	o	0	0	0
2	40	-1	1	0
6	67	8	6	7
9	74	30	32	31
14	81	51	60	56
20	81	64	73	69
23	86	78	80	79
28	88	74	84	79
29*	89	77	84	82

*measurement after acidification

in Table 3. A final mean value of 79 % has been determined, and in general, both sets perform quite in parallel.

Though one set seems to lag behind the other from day 9 ownwards. Taking the 10 day window as the acceptance criteria for “ready biodegradability”, Figure 2 b visualizes the biodegradation progress. Clearly again, the 60% level is reached within the prescribed time window, and we can consider DINP as readily biodegrable. Results from Sugatt et al. (1984), give high biodegradation rates for all the three mentioned phthalates. However, no clear cut results could be drawn from their paper, whether or not the substances tested were readily biodegradable. In order to be classified as readily biodegrable, special test systems have to be employed. The modified Sturm test belongs to this group and is especially designed for testing of poorly water soluble substances. A low density of the inoculum, which must originate from a non-industrial sewage treatment plant shall resemble the

low density of microorganisms of surface waters. In combination with a sufficient speed in the biodegradation process, it may be assumed, that substances passing the 10 day window will rapidely degrade in the environment (OECD, 1993).

Conflicting results, which show some phthalates not easily biodegradable (compilation in Lundberg, 1994), may result from inappropriate test designs. Test systems in which the biovailability of the test substance cannot be demonstrated or guaranteed, will of course show a poor biodegradation behavior. However, a substance not bioavailable cannot be attacked biologically. This will lead to false negative results, and thus to a wrong interpretation. A proper test design is especially necessary for poorly water soluble substances. The modified Sturm test employed here is adequate in this respect.

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