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THE NEED FOR APPLYING STABILITY TESTS IN BIODEGRADABILITY ASSESSMENTS

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ABSTRACT

Out of fourteen chemicals tested, two - hexamethylenetetramine and sodium benzene sulphinate - were degraded in screening tests (>70% DOC removal, >60% ThOD exerted) but not in activated sludge simulation tests (<25% DOC removal). It is suggested from data in the literature that these essentially non-biodegradable chemicals undergo hydrolysis or autoxidation in the 28-day screening tests to form biodegradable products, which are not produced in sufficient quantity in the 3-hour simulation test. The need is stressed for taking account of the stability of chemicals in water when assessing biodegradability in the environment.

INTRODUCTION

The accepted sequential system of methods for assessing the biodegradability of organic chemicals is enshrined in the Report of the OECD Chemicals Group (1). The scheme has been adopted in the UK (2); the methods have been accepted by the EEC (3) while the scheme is under discussion.

The hierarchy of testing depends on the principle that if a chemical is biodegraded in one of the first tier (screening) tests, the conditions of which are stringent, it is assumed to be readily biodegraded in the aquatic environment, including sewage treatment. (If on the other hand a chemical is not degraded in the screening test, it cannot be concluded that it will not degrade in the environment; other tests in the sequential scheme should be applied.)

In a recent calibration exercise ('ring test') of the EEC Respirometric Method (4) (to be published), fourteen chemicals, selected because difficulties had been encountered in screening tests, were examined. The method is similar to the Japanese MITI Method (5) but uses an activated sludge inoculum instead of one grown on a glucose-peptone-phosphate medium.

In a follow-up to that exercise, the same chemicals were examined at this Laboratory for their behaviour in the ISO Die-Away test (6) and in the activated sludge simulation test (7). The behaviour of ten of these chemicals in the simulation tests was consistent with that in the screening tests, that is, if they were degraded in screening tests they were removed in the simulation test. Inconsistent behaviour of two other chemicals, tetrahydrofuran and N-methylpyrrole, could be explained by virtue of their volatility, but the lack of degradation of the apparently readily biodegradable hexamethylenetetramine and sodium benzene sulphinate could not be so explained. This paper describes the discrepancies, offers an explanation for them and reinforces the need in some circumstances for physico-chemical stability tests.

METHODS AND MATERIALS

Chemicals. Benzene sulphinic acid, sodium salt, NaBS ($C_6H_5SO_2Na$) was obtained from Aldrich Chemicals Ltd., Gillingham, Dorset, UK. Hexamethylenetetramine, Hex, ($C_6H_{12}N_4$) was obtained from Aldrich Chemicals Ltd., and also from British Drug Houses Ltd., Dagenham, Essex, UK and from Fisons Ltd.

Screening Methods. The dissolved organic carbon (DOC) die-away test as described in the ISO method (6) was used; initial DOC concentrations were 10-50 mg/l and inocula concentrations were either 0.5 ml effluent/l or 30 mg activated sludge solids/l. Both the MITI (5) and EEC (4) versions of the respirometric were employed, using 100 mg test substance/l and 30 mg suspended solids/l as inoculum. Tests were carried at either 20 or 25 ^{o}C.

Simulation Methods. The activated sludge simulation test was carried out as described by the EEC (7). Both Husmann and WRc porous pot units were used in the direct non-coupled mode treating Stevenage settled domestic sewage at a retention time of 3 h and a sludge age of 6 d. These conditions resulted in concentrations of suspended solids in the activated sludge mixed liquor of 3000-4000 mg/l. In most experiments the temperature was kept in the range 18-25 ^{o}C, but in some it was deliberately maintained at 25 ^{o}C.

Carbon Analysis. In most experiments the Dohrmann DC80 carbon analyser was used: in a few, earlier tests the Erba Science TCM/480 had been employed.

RESULTS

The results of screening tests at this laboratory are given in Tables 1-3; it can be seen that in the presence of mercuric chloride, no removal of DOC occurred. They show that, whereas neither chemical was adequately removed in the 28-day period when the inoculum was sewage effluent, both Hex and NaBS were removed to a much larger extent when activated sludge was used as the inoculum

(Tables 1 and 2). High removals were also obtained in the two respirometric tests (Table 3).

However, while the necessary 60% of the theoretical oxygen demand (ThOD) and 70% DOC removal in a '10-day window'* was achieved on most occasions (8 out of 12; Tables 2 and 3) for NaBS, Hex was thus removed on only 3 out of 18 occasions (Tables 1 and 3).

After the full 28 days in the ISO test, Hex was degraded fully on all 12 occasions (Table 1) and NaBS on 5 out of 6 (Table 2), when sludge was the inoculum. In the respirometric method (Table 3) NaBS was adequately degraded in all 6 tests, but there was some variation with Hex; DOC showed 7 out of 8 values >70%, while only 6/12 individual ThOD values were over 60%.

In the statutory 9-week simulation tests, the removal of Hex did not exceed 21% DOC (Table 4) and removal was not increased when the temperature was maintained at 23-25 ^{o}C rather than at 18-20 ^{o}C (Table 4) nor when the experiment was extended for a further week. Removal of NaBS was slightly higher, at 27-30% DOC (Table 4).

For comparison it is of interest to note that in the EEC calibration exercise of the respirometric method (to be published), 8 out of 25 laboratories reported 28-day ThOD values greater than 60% for Hex (5 out of 21 gave >70% DOC removal), and 16 out of 21 reported >60% ThOD (14 out of 15 found >70% DOC removal) for NaBS.

DISCUSSION

The difficulty of removing hexamine both in laboratory-scale biological filters and activated sludge plants, on the one hand (8), and in full-scale sewage treatment (9) on the other, has been known for some time. The results, reported here, with Husmann and porous pot units have confirmed this.

It is tempting to postulate, as an explanation for the difference in results between screening tests and simulation tests, that the two substances can be attacked when individually present as sole sources of carbon and energy, but that in competition with the other substrates present in sewage some repressive or competitive biochemical mechanisms intervenes, leading to sequential oxidation. Thus, in the short time (3 h) available in the simulation method little of the test substance can be biochemically oxidised.

Other explanations , however, may more likely be correct: it is known than

* Strictly, for a chemical to be classified as 'readily biodegradable', it should be removed by the stated proportions in the 10 day period after 10% ThOD or DOC has been attained.

Hex hydrolyses in aqueous solution to ammonia and formaldehyde and that sulphinic acids are unstable in solutions, being oxidised to sulphonic acids. During the 28 days of the screening test, the original substances may be transformed sufficiently rapidly to their products, which are fairly readily biodegradable, to cause most of the DOC to disappear. In the simulation method, the stock solutions feeding the activated sludge units were made up weekly so that the 7 days (or average 3.5 d) plus 3 h in the aeration tanks would not have been long enough to allow formation by physico-chemical processes of sufficient amounts of biodegradable products, formaldehyde and benzene sulphonic acid.

Little data on the rate of hydrolysis of Hex could be found in the literature, but Strom and Won Jun (10) reported that its half-life period at 37.5 ^{o}C was as low as 1.6 h at pH 2 and 13.8 h at pH 5.8. It may be calculated from these data (10) and those of Tada (11) that at 30 ^{o}C the half-life period at pH 7.0 is as high as 160 days. Thus, the half-life period changes rapidly between pH 6 and 7. Also, Tada (11) reports that the rate of hydrolysis was increased in the presence of nitrite, which is sometimes formed during screening tests as a product of oxidation of ammonium salts. Although these calculated half-lives are high, they may go at least some way to explain the high removal of DOC in the screening tests and perhaps the removal of the formaldehyde by micro-organisms increased the rate of hydrolysis of Hex.

No quantitative data for the stability of solutions of NaBS could be found.

These 'false positive' screening-test results could lead to doubtful hazard assessments. Such results could be avoided by applying the 'Hydrolysis at 3 pH values' method (12), which would account not only for hydrolysis but also for other molecular changes, if the test applied is specific for the original test substance. Since the hydrolysis method can be time-consuming and costly, and since so few 'new' chemicals are likely to be readily biodegradable, it is suggested that it be applied after carrying out the screening test and only to chemicals which show positive 'removal' results.

If a chemical is unstable in solution, the products should be tested for biodegradability and a calculation should be made as to whether the hydrolytic products will be formed under the conditions prevailing in simulation tests. This is the reverse of the situation for testing for inhibition to bacteria. As so few 'new' chemicals are likely to be readily biodegradable, the first test applied should always be inhibition to bacteria to save wasted effort in carrying out a biodegradability assessment at inhibitory concentrations.

The manufacturers of a new chemical are likely to have determined its stability in solution when assessing it for the designed purpose, so that the problem described here is not likely to occur frequently with new chemicals.

Table 1. Removal of Hexamethylenetetramine (as DOC) in ISO Die-Away Tests

Inoculum	Initial concentration of Hex (mg DOC/1)	% Removal of DOC after (days) 7	14	21	28	Estimated* time for removal of 70% (days)
None, plus $HgCl_2$	15	-2	+4	-4	-2	-
None	15	4	9	26	47	-
Effluent (0.5 ml/1)		5	10	36	46	-
		4	4	-	31	-
		8	11	-	27	-
	10-15	8	17	-	21	-
		7	6	-	41	-
		7	13	-	18	-
		7	19	-	13	-
Effluent (10 ml/1)	15	4	15	30	62	-
MITI 'sludge'	15	0	51	75	103	11
(30 mg/1)	50	10	32	81	79	12
Activated sludge	10	4	64	95	97	8
(30 mg/1)	10	26	48	69	76	19
	14	65	82	99	108	9
		20†	76	-	93	10
		20	65	-	97	12
		24	66	-	88	12
	15	7	22	-	69	-
		6	27	-	88	16
		7‡	39	-	90	16

Each value is the average of 2 or 3 determinations
Sludges grown on Stevenage (UK) domestic sewage, except
† taken from Wargrave treatment works and
‡ taken from High Wycombe treatment works
* time from 10% to 70% DOC removal

Table 2. Removal of Sodium Benzene Sulphinate (as DOC) in ISO Die-Away Tests

Inoculum	Initial concentration of Hex (mg DOC/1)	% Removal of DOC after (days) 7	14	21	28	Estimated* time for removal of 70% (days)
None	15	0	6	11	16	-
Effluent (0.5 ml/1)	15	0	20	-	15	-
	15	5	21	-	9	-
MITI 'sludge'	10	0	20	102	98	8
(30 mg/1)	22	0	80	93	94	5
Activated sludge	10	0	18	76	94	10
(30 mg/1)	15	5†	32	-	20	-
	15	5‡	47	-	71	20
	22	0	13	35	85	13

Each value is the average of 2 or 3 determinations
Sludge grown on Stevenage (UK) domestic sewage, except
† taken from Wargrave treatment works
‡ taken from High Wycombe treatment works
* time from 10% to 70 DOC removal

Table 3. Removal of Hexamethylenetetramine and Sodium Benzene Sulphinate in Respirometric Tests

Method	Delay time t_d* (days)	% ThOD* after (days) $(t_d + 10)$	% ThOD* after (days) 28	% DOC removed at 28 days
Hexamethylenetetramine				
EEC	0, 0	15,17	59,62	94,96
	3, 3	13,20	27,59	nd
	3, 3	22,41	82,87	97,98
MITI	7, 1	14,23	38,67	nd
	3, 7	6, 8	46,74	97,97
	13,13	31,43	46,70	54,83
Sodium Benzene Sulphinate				
EEC	4	50	68	98
	5	63	70	nd
	11	63	68	100
MITI	5	70	73	97
	7	83	95	nd
	11	69	79	97

* assumed that nitrate was produced at $(t_d + 10)$d; known to be produced at 28 d
Values for Hex. are single determinations: for NaBS values are averages of duplicates
nd = not determined

Table 4. Removal of Hexamethylenetetramine and Sodium Benzene Sulphinate in Activated Sludge Simulation Tests
(Non-coupled units; sludge age = 6 d)
(Total time of experiment = 9 weeks)

Type of vessel	Temperature (°C)	Concentration of test substance (mg C/l)	% Removal - mean over final 3 week period (n = 15) 1	2
Hexamethylenetetramine				
Husmann	18-20	17.5	21 (11.9)	21 (11.1)
Porous pot	18-20	20	16 (13.1)	21 (12.6)
Porous pot	23-25	20	12 (11.2)	17 (11.2)
Sodium Benzene Sulphinate				
Husmann	18-20	20	27 (17.6)	30 (19.1)

1,2 are duplicate vessels
Values in brackets are standard deviations

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