

Results of a “Whole Effluent Assessment” study from different industrial sectors in Germany according to OSPAR’s WEA strategy

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Supplementary Material

Results of non-target GC/MS- screening of the effluent samples under investigation (after solvent extraction or stir-bar sorptive extraction)

The wastewater samples A, C, D, and E were sent to the RIZA (Netherlands Institute for Inland Water Management and Waste Water Treatment) and screened for the presence of organic substances as have been reported in the report of the OSPAR practical study programme 2003¹. Briefly, the samples were extracted using a liquid-liquid extraction with dichloromethane. The organic substances in the extract were separated using gas chromatography with mass spectrometry (GC-MS) together with the peak identification according to the latest version of the AMDIS software and the NIST98 library on mass-spectra.

In the following tables 1-4 (shorten from the above mentioned report) we have included only such compounds which were identified with a high level of confidence. (The authors of this report remarked that for many additional substances, identification is unsure and that it is unknown how many substances are left unidentified.)

Table 1: Wastewater “A”- textile industry

Identified Substance	CAS No.
m-pyrol	872-50-4
9,10-anthracenedione	84-65-1
ethanol, 2-(2-ethoxyethoxy)-	111-90-0
toluidine (o-), 5-chloro-	95-79-4
diisobutyl phthalate	84-69-5
dimethyl phthalate	131-11-3
triisobutylphosphate	126-71-6
ethanol, 2-butoxy-	111-76-2
phthalic acid, diethyl ester	84-66-2
benzene, 1-chloro-2-nitro-	88-73-3
2-propanol, 1-butoxy-	5131-66-8
hexanoic acid, 2-ethyl-	149-57-5
isoquinoline	119-65-3
ethanol, 2-butoxy-	111-76-2

¹ A. Gerritsen et al., OSPAR practical study programme 2003 on Whole Effluent Assessment (WEA), in: OSPAR Commission (ed.), Whole Effluent Assessment Report, 2005 (ISBN 1-904426-45-X); see: http://www.ospar.org/documents/dbase/publications/p00219_WEA%20report.pdf

Table 2: Wastewater “C” - pharmaceutical industry

Identified Substance	CAS No.
diisobutyl phthalate	84-69-5
triisobutylphosphate	126-71-6
hexanoic acid, 2-ethyl-	149-57-5
9,10-anthracenedione	84-65-1
toluene, a-chloro-	100-44-7
limonene	138-86-3
pyrrolidinedion(2,5), 1-methyl-	1121-07-9
toluidine (o-), 5-chloro-	95-79-4
phthalic acid, diethyl ester	84-66-2
toluene, 3-chloro-	108-41-8
benzenamine, n,n-diethyl-	91-66-7
acetophenone	98-86-2
benzenesulfonamide, N-butyl-	3622-84-2

Table 3: Wastewater “D” - chemical and pharmaceutical industry

Identified Substance	CAS No.
diisobutyl phthalate	84-69-5
triisobutylphosphate	126-71-6
hexanoic acid, 2-ethyl-	149-57-5
heptanoic acid	111-14-8
phthalic acid, diethyl ester	84-66-2
acetophenone	98-86-2
butylated hydroxytoluene	128-37-0
limonene	138-86-3
nonane	111-84-2
nonanal	124-19-6
alpha-Methylstyrene	98-83-9
tributyl phosphate	126-73-8
heptane, 2,3-dimethyl-	3074-71-3
hexane, 2,3,3-trimethyl-	16747-28-7
benzothiazole	95-16-9
butane, 2-methoxy-2-methyl-	62016-49-3
acetic acid, 1-methylethyl ester	108-21-4
tridecane	629-50-5
styrene	100-42-5
phthalic acid, benzyl butyl ester	85-68-7
tris(2-chloroisopropyl)phosphate	13674-84-5
dimethyl phthalate	131-11-3
octane	111-65-9
hexadecane	544-76-3
cyclohexane, 1,2,3-trimethyl-	1678-81-5
benzene, 1,2,4-trimethyl-	95-63-6
n-heptacosane	593-49-7
stearineacid (octadecanoic acid)	57-11-4
docosane)	629-97-0
octadecane	593-45-3
cyclohexane, 1,3,5-trimethyl-	1839-63-0
benzenesulfonamide, N-butyl-	3622-84-2

Table 4: Wastewater “E” - textile finishing industry

Identified Substance	CAS No.
diisobutyl phthalate	84-69-5
triisobutylphosphate	126-71-6
hexanoic acid, 2-ethyl-	149-57-5
phthalic acid, diethyl ester	84-66-2
acetophenone	98-86-2
benzothiazole	95-16-9
limonene	138-86-3
alpha-methylstyrene	98-83-9
tributyl phosphate	126-73-8
formamide, N,N-dibutyl-	761-65-9
butylated hydroxytoluene	128-37-0
2,4,7,9-tetramethyl-5-decyne-4,7-diol	126-86-3
phthalic acid, benzyl butyl ester	85-68-7
butane, 2-methoxy-2-methyl-	62016-49-3
acetic acid, 1-methylethyl ester	108-21-4
styrene	100-42-5
tris(2-chloroisopropyl)phosphate	13674-84-5
furan, tetrahydro-2,5-dimethoxy-	696-59-3
n-heptacosane	593-49-7
benzene, 1,2,4-trimethyl-	95-63-6
Hexadecane	544-76-3
benzene, propyl-	103-65-1

The wastewater samples B, F ,G and H were analysed in the Department of Ecological Chemistry at the UFZ Leipzig, Germany. Two very different extraction methods were carried out with sample aliquots.

The first method was a liquid-liquid extraction following the ‘EGOM’ LLE procedure as redrafted by Leslie and Leonards². Here, 300 ml of wastewater were first acidified (pH<2) and extracted twice with 30 ml cyclohexane. Then the sample pH was adjusted to >10 and two more extractions with 30 ml cyclohexane were performed. All four extracts were combined, dried and reduced to 2 ml. After liquid injection of 1 µL into an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a mass spectrometric detector (MSD) 5973N, the substances were separated on a DB-5MS capillary column (60 m × 0.25 mm I.D, 0.25 µm film thickness). The injector temperature was 250°C. The GC oven temperature program was: 60°C for 1 min, ramped at 30°/min to 150°C, then at 6°/min to 180 °C, and finally at 4°/min to 280°C, hold for 16,5 min. The temperature of transfer-line and detector was 280 °C. Helium 5.0 was the carrier gas with a constant flow velocity of 1.3 mL/min. MS-Data acquisition was performed in SCAN mode with a solvent delay time of 6 min and scanning of ion masses 20 to 350 from minute 6 to 15 of the run and of masses 20 to 550 after 15 min. Substance assignment to the main peaks was performed using the MS library NIST-05, version 2.0, and only compounds with a match factor > 0.8 were reported.

Secondly aliquotes of the samples were subject of stir-bar sorptive extraction (SBSE) with Twister bars (Gerstel, Mülheim a.d.R., Germany). The microextraction conditions were chosen as follows: 100 mL of the sample were put together with a Twister bar in an approx. 100-mL Erlenmeyer flask, so that practically only a small headspace was left. Then the sample was stirred with the Twister bar for 48 h at 800 r.p.m. with a multiplace magnetic

² H.A. Leslie, P.E.G. Leonards: Protocol Determination of bioaccumulative substances in whole effluents using the ‘EGOM’ liquid-liquid extraction method. OSPA-IEG on WEA Interlaboratory Study 2005, RIVO, Ymuiden, NL 2005.

stirrer Variomag (H+P Labortechnik, Oberschleißheim/Germany). The Twister bars were taken out of the samples, shortly dried with tissue paper, put individually in small GC vials and stored in the freezer (at. -20°C) until further processing.

The Twister bars were thermally desorbed followed by gas chromatographic separation and mass selective detection. Thereby a thermodesorption unit (TDU) from Gerstel which is placed on top of an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a cold injection system CIS-4 (Gerstel) and a mass spectrometric detector (MSD) 5973N (Agilent) was used. A MPS-2 xyz robot with a Twister tray (both from Gerstel) allowed automated desorption of the Twister bars in series. While waiting in the tray for processing, the sample liners were held at approx. 10 °C by cooling the underlying aluminium block with a cryostat RE 104 (Lauda, Königshofen, Germany). For thermal desorption of Twisters in splitless mode the TDU was programmed from 30 °C (1 min) to 280 °C (5 min) at a rate of 12 °/s. The transfer temperature was set to 330 °C. The analytes were cryo-focused in the CIS at -10 °C with liquid nitrogen prior injection. For splitless injection the CIS was ramped from -10 °C (7 min) at a rate of 12 °/s to 300 °C (5 min).

The substances were separated on a DB-5MS capillary column (60 m × 0.25 mm I.D, 0.25 µm film thickness). The injector temperature was 250°C. The GC oven temperature program was: 60 °C for 5 min, ramped at 10 °/min to 180 °C, then at 8 °/min to 280 °C, hold for 29 min. The transfer-line and detector temperature was 280 °C. Helium was the carrier gas with a constant flow velocity of 1.1 mL/min. MS-Data acquisition was performed in SCAN mode with a solvent delay time of 6 min and scanning of ion masses 20 to 350 from minute 6 to 15 of the run and of masses 20 to 550 after 15 min. Substance assignment to the main peaks was performed using the MS library NIST-05, version 2.0, and only compounds with a match factor > 0.8 were reported.

The results are summarised in table 5.

Table 5: Analytical results with wastewaters “B”, “F”, “G”, and “H”

(b = before and a = after degradation test; S = SBSE; grey background of cell = LLE)

Identified Substance	CAS No.	B		F		G		H	
		b	a	b	a	b	a	b	a
1-Phenoxypropan-2-ol	770-35-4	grey							
Nonanoic acid	112-05-01			grey					
1'-Bicyclohexy	92-51-3			grey					
Ethanol, 2-(2-butoxyethoxy)-, acetate	124-17-4	grey							
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	126-86-3	grey							
Benzoic acid, p-tert-butyl-	98-73-7	grey		grey	grey				
Decanoic acid, 3-methyl-	60308-82-9	grey							
Benzoic acid, p-tert-butyl	98-73-7			grey					
1,2-Benzenedicarboxylic acid, diethyl ester	84-66-2							grey	
Benzenamine, 2-methoxy-4-nitro-	97-52-9			S	grey				
9-Hexadecenoic acid	2091-29-4	grey							
Heptacosane	593-49-7		grey						
Dibenzofuran, 1,3,7-trichloro-	64560-16-3								
Thiocyanic acid, bicyclo[4.4.1]undeca-1,3,5,7,9-pentaen-2-yl ester	115163-01-4								
4,4'-Dimethoxybenzophenone	90-96-0				grey				
1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester	85-68-7			S	grey	S	S	grey	
Hexanedioic acid, dioctyl ester	123-79-5		grey					grey	
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	119-47-1							grey	
di-2-Ethylhexyl chloroformate	24468-13-1				grey				
Triphenylphosphine oxide	791-28-6	grey	S						
2-Ethylhexyl chloroformate	24468-13-1					grey			grey
Erucylamide	112-84-5					grey		grey	
Cholest-5-en-3-ol (3.beta.)-	57-88-5				grey			grey	grey