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RESEARCH ARTICLE

Do cemeteries emit drugs? A case study from southern Germany

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Abstract

The risk of earth burials for the environment and public health is a matter of controversial debate. The aim of the present study is to characterise the drainage of cemeteries with regard to the concentration of a number of pharmaceuticals and to the soil's hydrochemical properties, and to discuss these data in comparison with data obtained for surface waters located upstream of the cemeteries. Of the 12 drainage samples analysed using LC-ESI-MS/MS, seven contained carbamazepine (< 225 ng l^{-1}), five contained hydrochlorothiazide, one contained metoprolol (23 ng l^{-1}) and one contained traces of ibuprofen. The surface water samples contained a larger number of different drugs (8 of the 12 drugs under investigation) and higher concentrations (e.g. metropolol 2230 ng l^{-1}). The NO_3, NH_4, PO_4 and DOC concentrations and the electrical conductivity of the cemetery drainages were in several samples higher than those of the surface water samples. The NO_3 and NH_4 concentrations exceeded the legal contaminant limits of drinking water in only one case. The present study found that the release of drugs and nutrients from cemeteries, measured in surface water drug loads, presents a low environmental risk. However, the study is only a snapshot and long-term monitoring of cemetery drainages, including a broad range of pharmaceuticals and detailed hydrological investigations, will have to be carried out before more substantiated statements can be made.

Keywords Pharmaceutical compounds · Cemeteries · Surface water · Water quality

Introduction

The risk of earth burials for the environment and public health is a matter of controversial debate. The opinion that cemeteries are residue landfills (Dent and Knight 1998) seems justified as earth burials bring into the soil nutrients as well as organic carbon (Mattern et al. 2011; Zychowski 2011; Majgier et al. 2014), heavy metals (Uslu et al. 2009; Jonker and Olivier 2012; Amuno 2013), trace elements (Amuno and Amuno 2014), burial-related artifacts (e.g. polyester textiles that decompose only with difficulty) and coffin upholstery (Fiedler et al. 2012) as well as artificial joints, cardiac pacemakers and jewellery (Berryman et al. 1991).

In addition to focusing on the enrichment of certain elements and substances in cemetery soil (necrosol), cemetery-related discussions also revolve around the potential contamination of groundwater and adjacent surface waters with phenols and formaldehydes (Zume 2011), especially nitrogen (nitrate, ammonium) and pathogenic germs (Pacheco et al. 1991; Martins et al. 1991; Nas and Berktay 2010; Oliveira et al. 2013; Fineza et al. 2014; Zychowski and Bryndal 2015). Elevated electrical conductivities observed in cemetery outflows are an indication that substances have been discharged from graves (van Haaren 1951; van der Honing et al. 1988; Pacheco et al. 1991). Investigations carried out by Dent and Knight (1998) and Żychowski (2012) show that elevated conductivities are not always down to the fact that the area is used as cemetery.

Paíga and Delerue-Matos (2016) investigated five Portugese cemeteries where the concentration of pharmaceutical substances in the tap water samples originated directly from the groundwater of these graveyards. All samples contained carbamazepine (20 ng l^{-1}), albeit in concentrations well below the legal contaminant standards for drinking water (42 ng l^{-1}, Vulliet and Cren-Olive 2011).

Despite the popularity of the subject in the media, there do not seem to be any studies that, in addition to focusing on water chemistry aspects, have concentrated specifically on

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pharmaceutically active substances in cemetery drainages. In view of the increasing quantities of medical drugs consumed (OECD 2014), higher life expectancy with simultaneously increasing population numbers and the high persistence of many pharmaceutically active substances in the environment, it seems plausible that pharmaceutical drugs are released through graveyard drainage. Forensic case studies show that pharmaceutically active substances can be detected in corpses for long periods of time (Thieme et al. 2006; Gaillard et al. 2011; Wollersen et al. 2014). Since most drugs are stable and polar (Ternes 2005), it seems also probable that, in addition to normal decomposition products, drug residues enter cemetery drainage as corpses decompose.

Our study aims to characterise drainage samples from representative cemeteries with regard to the concentration of selected pharmaceutically active substances and hydrochemical properties, as well as to compare them with surface water (streams) located upstream of the cemeteries from where the drainage samples were taken.

Our hypotheses are:

(1) Pharmaceuticals are detectable in drainage water, but the concentrations are much lower compared to the respective concentrations in surface water.
(2) Drainage has higher nitrate (NO_3), ammonium (NH_4), phosphate (PO_4) and dissolved organic carbon (DOC) concentrations and higher electric conductivity than surface waters.

Material and methods

Cemeteries in regions of Germany that are characterised by the consumption of relatively high quantities of medicines were chosen for the investigation (GERMAP 2012). The selection was based on an evaluation of cemetery reports ($n = 260$). The selection criterion was the recommendation of experts to install drainage systems in order to improve conditions in the cemeteries ($n = 54$). Drainage systems were implemented in less than 50% of the cemeteries. Of these cemeteries, 20 were suitable for the present study and samples were taken between April and June 2012. The drainages of eight cemeteries did not contain any water during the sampling period. The study therefore involved samples from 12 cemetery drainages and the respective streams located upstream of the cemeteries.

In the cemeteries investigated, burial field and burial chamber drainages were installed between 1985 and 2010. The selected graveyards are located in areas of differently sized source rock that is typical of the region (Table 1). With the exception of one cemetery where the last burial took place in 1987 (one grave), the last burial took place no longer than 12 months prior to sampling. The typical resting time is between 15 and 35 years.

Twelve drugs of different classes were included in the analysis (Table 2). The drainage samples were taken from drainage wells located closest to the drainage area (around 2.5 m below the soil surface): In the graveyards under investigation,

Table 1 Study sites under investigation

Cemetery	System	Installation of drainge system (year)	Drainage area (m^2)	Grave numbers	Last burial	Parent material	Resting time(a)
1	Single grave	–	2	1	1985	Physically preweathered periglacial regolith of Buntsandstein (Triassic Red Sandstone)	28
2	Grave field drainage	2010	473	17	2012	Quartary sand and gravel top layers	25
3	Grave field drainage	2006	unk.	74	unk.	Physically preweathered periglacial regolith of Buntsandstein	25
4	Grave field drainage	unk.	unk.	unk.	unk.	Physically preweathered periglacial regolith of Buntsandstein	25
5	Grave chamber drainage	2003	1500	28	2012	Early Pleistocene river deposits containing Loess soil	15
6	Grave field drainage	1987	5000	357	2012	Loess over Buntsandstein	35
7	Grave chamber drainage	2000	700	110	2011	Loess over Devonian bedrock	25
8	Grave chamber drainage	2002	1100	150	2012	Physically preweathered periglacial regolith over Devonian bedrock	25
9	Grave chamber drainage	2003	12,000	760	2011	Physically preweathered periglacial regolith over Devonian bedrock	35
10	Grave field drainage	1989	unk.	387	unk.	Early Pleistocene river deposits containing Loess	25
11	Grave field drainage	unk.	unk.	unk.	unk.	Physically preweathered periglacial regolith over Rotliegend	25
12	Grave field drainage	2007	10,000	300	unk.	Loess over Buntsandstein	25

unk. unkown

drainage systems were introduced at a depth of 2.6 to 3 m below burial grounds and major walking paths. The water collected was drained off via drainpipes. Water was sampled in the inspection shafts at a depth of around 3.5 m. The shafts were located downstream of the direction of drainage. The sampling containers were immersed into the feeding strands to remove the water. The samples were taken in April 2012. Precipitation (between 35 and 70 mm) was much lower than the long-term average (30%). Nevertheless, each inspection shaft contained flowing water from which samples could be withdrawn. In order to exclude contaminations, the sampling vessels (brown glass bottles, 3 × 1 l) were rinsed three times with drainage water prior to collecting the water samples that were used for the current investigation.

The water samples were transported to the laboratory in cooling boxes and extracted the following day using solid-phase extraction (SPE) (Strata-X cartridges, 33 μm, 85 Å, polymeric RP: 2 g 20 ml^{-1}, Giga tubes, Phenomenex, USA) and enriched for subsequent measurements. The Strata-X cartridges were conditioned with 10 ml *n*-hexane, 10 ml ethyl acetate, 20 ml methanol and finally 20 ml of distilled, deionised water. The water sample (2000 ml) was percolated through the cartridge without vacuum. After drying the cartridge for 6 h with nitrogen, the analytes were eluted with 2 × 5 ml *n*-hexane, 2 × 5 ml ethyl acetate and 3 × 5 ml methanol without vacuum. This eluate was evaporated to dryness at 50 °C with a Pierce Reacti-Therm 18780 heating module (Piece, Rockford, USA). Methanol was evaporated first, then *n*-hexane and finally ethyl acetate. The residue was dissolved in 100 μl buffer solution (0.1% formic acid + 5 mmol ammonium formate in 1 l distilled, deionised water). Ten microlitres was injected directly into the gas chromatograph. The analyte recovery rates ranged between 70 and 110%.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses were performed using reverse-phase liquid chromatography coupled to tandem mass spectrometry. Liquid chromatography was performed on an HP1200 HPLC system (Agilent Technologies) coupled to a triple-stage quadrupole mass spectrometer (AB Sciex API 4000) with an electrospray ion source (TurboV). The chromatographic separation was achieved on a Zorbax Eclipse XDB-C18 column (3 × 150 mm, 3 μm; Agilent Technologies) using mobile phase A (water, 5 mM ammonium formate; pH 3.5) and mobile phase B (methanol, 5 mM ammonium formate; pH 3.5) in a gradient programme with a total flow of 850 μl min^{-1} (binary system): 0–1 min: 90% A; 1–9 min: 90% A to 0% A; 9–14 min: 0% A; 14–16 min: 0% A to 90% A. Individual transitions of the molecular ions ([M+H+]) were detected in positive and negative multiple reaction monitoring mode (MRM), listed in Table 2 along with the fragmentor voltages, declustering potentials and detection limits. For quantification, peak area ratios of the analytes and the internal standard (2-methyl-1-phenyl-2-propylhydroperoxide) were calculated as a function of the substance concentrations.

Electrical conductivity (Cond 340i, Xylem Analytics Germany Sales GmbH & Co. KG, WTW, Weilheim, Germany) and pH value (inoLab pH 720, Xylem Analytics Germany Sales GmbH & Co. KG WTW, Weilheim, Germany) were determined on site. Nitrate (NO_3), ammonium (NH_4), phosphate (PO_4) and dissolved organic carbon (DOC) concentrations were determined following filtration (nitrate-acetate and cellulose-acetate membrane filters (< 45 μm) using a flow injection analysis system (FIAstar ^{TM} 5000, FOSS Analytical, Denmark) and a UV/VIS photometer (Specord 50 Plus, Analytik Jena, Germany) and TOC analyser (high TOC II, Elementar, Hanau, Germany). All measurements were carried out in duplicate.

Results and discussion

Concentrations of pharmaceutically active substances

Of the drugs investigated, it was possible to determine the quantity of atenolol, metaprolol, carbamazepine, indomethacin, naproxen and diclofenac. In the case of hydrochlorothiaxide and ibuprofen, negative ion mode mass spectrometry proved to be more efficient, sensitive and selective than positive ion mass spectra (Table 2). While the spectra of the two drugs could be produced, it was impossible to determine their quantity. The trimethoprim, propranol, chlorthalidon and furoslemid concentrations of all samples were below detection limits (Table 2).

Four of the 12 drugs of interest were detected in the cemetery drainages investigated (Table 3). Two drugs were detected in three separate samples and one drug in six different samples. Seven different drugs were identified in the surface water samples; one sample contained six drugs, another five, another three, another two and two samples contained one drug each. No drugs at all could be identified in the drainage samples and surface water samples of four sampling sites.

The concentrations of carbamazepine, metoprolol, diclofenac and atenolol in the surface water samples were significantly higher than those of the drainages. In the surface water samples, 27% of the concentrations of the four drugs were above the detection limit, and 15% of the concentrations were in the 0.1–0.57 μg l^{-1} range. The maximum concentration was 2.2 μg l^{-1} (metoprolol). In comparison, in 16% of cases concentrations of the four drugs were above the detection limit and only 4% in the range between 0.1 and 0.225 μg l^{-1} carbamazepine, which degrades only with difficulty (Chefetz et al. 2008; Zhang et al. 2008), was present in 58% of all drainage water samples and in 41% of the surface water samples examined. The concentrations determined were in a concentration range that is generally reported in the literature for streams of comparable size, e.g. in a study on

Table 2 Ancillary information (registry number, CAS number; field of application; molecular weight, MW) (Wishart et al. 2006) and instrumental parameters of the LC-MS/MS analysis

Substance(CAS number)	Field of application	MW	RT	IM	Collision energy	Declustering potential	MRM transition	LOQ	LOD
		[g mol^{-1}]	(min)		(V)	(V)		(ng l^{-1})	(ng l^{-1})
Atenolol(29122-68-7)	(Beta 1-selective) beta blocker	266.34	3.27	PI	35	61	267–145	10	5
Metoprolol(37350-58-6)	(Beta 1-selective) beta blocker	267.36	5.52	PI	27	61	268–116	10	5
Propranolol(525-66-6)	(Non-selective) beta blocker	259.34	6.65	PI	27	36	260–116	20	10
Carbamazepine(298-46-4)	Anti-epileptic drug, also important anticonvulsant	236.27	7.37	PI	25	66	237–194	10	5
Chlorthalidone(77-36-1)	Analgestic	338.76	5.84	PI	13	76	339–322	10	5
Hydrochlorothiazide(58-93-5)	Analgestic	297.74	10.58	NI	− 26	− 75	296–269	10	5
Indomethacin(53-86-1)	Analgestic, antipyretic, also has anti-inflammotory effect	357.79	9.04	PI	27	66	358–138	10	5
Naproxen(22204-53-1)	Analgestic, anti-inflammotory agent, antipyretic	230.27	8.25	PI	19	61	231–185	10	5
Diclofenac(15307-86-5)	Non-opiod analgestic, arylacetic acid derivative	296.15	9.01	PI	29	21	296–215	10	5
Furosemid(54-31-9)	Diuretic	330.74	8.26	NI	− 20	− 28	329–285	10	5
Ibuprofen(15687-27-1)	Antiphlogistic, arylpropionic acid derivative	206.28	5.01	NI	− 13	− 28	205–161	10	5
Trimethoprim(738-70-5)	Antibiotic	290.32	4.45	PI	33	51	292–231	20	10

MW, molecular weight; *RT*, retention time; *IM*, ionisation mode; *PI*, positive ion mode; *NI*, negative ion mode; *MRM* (multiple reaction monitoring) transition; *LOD*, limit of detection; *LOQ*, limit of quantification

Austria, Ternes (2005) reported a maximum carbamazepine concentration of 294 ng l^{-1} (median 75 ng l^{-1}). The maximum carbamazepine concentration in the drainage samples was 225 ng l^{-1} (median 32 ng l^{-1}). Our finding clearly shows that carbamazepine remains chemically stable both during treatment in sewage treatment plants (Clara et al. 2004; Löffler et al. 2005; Fatta-Kassinos et al. 2011), and also in a decomposing corpse in earth graves.

The maximum carbamazepine concentration in surface waters was twice that of the drainage water samples (418 ng l^{-1}, median 247 ng l^{-1}). Higher carbamazepine concentrations were reported in larger streams (Zuehlke et al. 2004, up to 1.3 μg l^{-1}, rivers/streams in Berlin; Carmona et al. 2014, up to 3.4 μg l^{-1}, Turia River Basin, Spain; Sacher et al. 1998, up to 2.1 μg l^{-1}, Rhine). An octanol/water distribution coefficient (log K_{ow}) of between 2.25 and 2.45 (Mersmann et al. 2002; Jones et al. 2005) suggests low sorption. This assumption is substantiated by the low elimination of the drug during its passage through the subsoil (Drewes 2003). Carbamazepine has a high persistence and is thus ubiquitously detectable in the environment (Löffler et al. 2005). Stuart et al. (2012) reported carbamazepine concentrations of up to 3600 ng l^{-1} in groundwater. A concentration of up to 42 ng l^{-1} carbamazepine has been found in drinking water (Vulliet and Cren-Olive 2011). Carbamazepine is used as marker for determining the anthropogenic influence on aquatic systems (Ruzicka et al. 2011).

Hydrochlorothiazide was detected but not quantified in four of the drainage water samples investigated and in seven of the surface water samples investigated. After carbamazepine, hydrochlorothiazide is the second most common drug in our study. However, the concentration was much lower than that measured by Vulliet and Cren-Olive (2011) in Spanish groundwater.

Metoprolol was only present in one of the sampled cemetery drainages (23 ng l^{-1}) at a significantly lower concentration than the maximum concentration of the surface waters investigated (2230 ng l^{-1}). Ternes (2000) found metoprolol concentrations of up to 2200 ng l^{-1} in various rivers in Germany.

Diclofenac concentrations of between 129 and 574 ng l^{-1} (Table 3) were measured in three of the surface water samples investigated, but not in the drainage samples, which are in the mean range of the concentrations of surface/groundwaters given in the literature and somewhat below those of drinking water (Table 3). Atenolol and naproxen were detected in two of the surface water samples examined (57–301 and 41–81 ng l^{-1}), but not in the cemetery drainage water samples. While the concentration of atenolol was significantly higher than that given in the literature (Table 3), the naproxen concentrations were in the fluctuation range given in the literature (16–830 ng l^{-1}) for surface waters (Kunkel and Radke 2012).

In contrast to the ibuprofen concentrations in ground/surface water (Table 3), which are frequently mentioned in

Table 3 Results of graveyard drainage and surface water monitoring compared to literature results for selected pharmaceutical contaminants in Europe

Substance	This study				Literature data [reference]		
	Drainage in graveyards ($n = 12$)		Surface water ($n = 12$)		Surface water	Groundwater	Drinking water
	Positive findings (n) | *site*	Concentration, range (mean) (ng l^{-1})	Positive findings (n) | *site*	Range (mean) (ng l^{-1})	Range or maximum (ng l^{-1})		
Atenolol	*0*		2 |*6*, *8*	57–301	4.7[1]	5.5[1]	34[1]
Metoprolol	1 |*6*	23	2 |*5*, *6*	450–2230	5–440[2], 2200[3]	0.3[1], 9–56[4]	1–26[1]
Propranolol	0		0		35–107[5]		
Carbamazepine	7 | *2*, *4–6*, *9*, *11*, *12*	10–225 (85)	5 | *5*, *6*, *8*, *9*, *10*	43–418 (222)	< 20–2500[6]	3600[7], 1–83[8], 9–167[4]	10[1], 42[1]
Chlorthalidone	0		0				
Hydrochlorothiazide	4| *2*, *6*, *8*, *12*	✓	7 | *1*, *2*, *6–10*	✓		59–2548[4]	
Indomethacin	0		1 | *8*	67			
Naproxen	0		2 | *6*, *8*	41–81	278–7189[10], 16–830[2]	1.2[1]	6[9]
Diclofenac	0		3 | *6*, *8*, *9*	129–574 (314)	3500[10], 5–760[1], 420[12], 900[13]	60–219[4], 380[12]	56[9]
Furosemid	0		0				
Ibuprofen	1 | *7*	✓	0	–	226–2370[5], 36[14]	200[7]	14[1]
Trimethoprim	0		0				

✓ identified, but quantity could not be determined

[1] Vulliet and Cren-Olive (2011), [2] Kunkel and Radke (2012), [3] Ternes (1998), [4] Radjenovic et al. (2008), [5] Roberts and Thomas (2006), [6] Wiegel et al. (2004), [7] Stuart et al. (2012), [8] Osenbruck et al. (2007), [9] Vulliet et al. (2011), [10] Carmona et al. (2014), [11] Heberer et al. (1998), [12] Heberer (2002), [13] Sacher et al. (2008), [14] Moldovan (2006)

Table 4 Comparison of common chemical parameters of graveyard drainage with literature parameters

	pH	Electric conductivity Range (mean) µS cm^{-1}	NO_3 Range (mean) mg NO_3 l^{-1}	NH_4 Range (mean) mg NH_4 l^{-1}	PO_4 Range (mean) mg PO_4 l^{-1}	DOC Range (mean) mg l^{-1}
This study						
Drainage	6.6–7.5 (7.1)	155–1977 (618)	0.2–68 (14.9)	< 0.05–2.8 (0.4)	< 0.1–1.9 (0.3)	1.3–11 (5.5)
Surface water	6.4–8 (7.1)	45–1041 (307)	0.1–24 (8.6)	< 0.05–0.8 (0.15)	< 0.1–0.7 (0.13)	1.1–7.8 (4.0)
Literature data [reference] groundwater in graveyards and (control)						
[1]		725–3200	< 0.05	0.2–4.1		
[2]			5–28 (4–7)	1–5 (0.2–2)	0.2–3 (0.3–0.9)	
[3]		740 (194)	6.2 (6.1)	1.2 (0.1)	3.4 (0.9)	
[4]		216–2204 (60–1127)	0–33 (0.2–6.3)	0–4.7 (0–0.79)	0–4.7 (0–2.5)	0–30 (2–73)
[5]	6.5–7.9 (6.5–6.9)	14–1360 (75–134)	< 0.1–55 (< 0.1)	< 0.1–89 (< 0.1–2)	0.3–37 (2)	2–218 (0.1–10)
[6]			10	4–400		
[7]	6.0–7.0 (6.0–7.0)	750–1400 (120–280)	2–145 (0–2.2)	6–88 (0–3.8)		
[8]			75.5			
[9]				0.05–2.5		7–25
[10]	7.3	1542	84			
[11]		175–1000	0.04–433	0.25–9	0.03–0.3	
Drinking water (threshold)						
[12]		2500	50	0.5		

[1] van Haaren (1951), [2] Żychowski (2012), [3] Knight and Dent (1995), [4] Dent and Knigth (1998), [5] Engelbrecht (1998), [6] Young et al. (2002), [7] Fineza et al. (2014), [8] Martins et al. (1991), [9] Trick et al. (2005), [10] Nas and Berktay (2010), [11] Migliorini (1994), [12] Concil of European Union (1998)

the literature, ibuprofen was detected in only one of the drainages examined, but not in the surface water samples.

Water chemistry

In analogy to the pharmaceutical substances investigated, high variability in the hydrochemical parameters investigated was observed both in the cemetery drainage and the surface water samples (Table 4). In accordance with numerous studies (Pacheco et al. 1991; Trick et al. 2005; Fineza et al. 2014), the nitrate, phosphate, ammonium and DOC concentrations in the cemetery drainage samples were slightly higher than those of the surface/groundwater samples. They also had a higher electrical conductivity (Table 4). Żychowski (2012) observed that the groundwater had much higher hydrochemical values than the surrounding area and put this down to a mass grave from the Second World War (0.24 mg NH_4 l^{-1}, 4 mg NO_3 l^{-1}, 0.94 mg PO_4 l^{-1}) in Niepołomice (Poland) (1.11 mg NH_4 l^{-1}, 28 mg NO_3 l^{-1}, 2.9 mg PO_4 l^{-1}).

Dent and Knight (1998) also measured higher electrical conductivity and higher DOC concentrations in the groundwaters of a cemetery in Melbourne than in the immediate surroundings (608–2204 vs. 241–263 µS cm^{-1}, 2–4 vs. 0–30 mg DOC l^{-1}). In the same study, however, the authors also reported on investigations in a cemetery in Perth, which showed lower conductivities and DOC concentrations in cemetery groundwater in relation to a reference site (216–667 vs. 60–1127 µS cm^{-1}, 4–23 vs. 59 DOC l^{-1}). In this context, Żychowski (2012) points out that exact comparisons between a cemetery and reference sites are difficult to make due to strong geogenic substrate differences and inadequate hydraulic gradients. This highlights the difficulty of quantifying the potential ecological risk of cemeteries.

Conclusion

Our research shows that some of the drugs investigated are found in both surface waters and cemetery drainages. In all water samples examined, the concentrations of pharmaceutically active substances of the cemetery drainages were always lower than those of the surface water samples.

The water samples investigated were taken during a rather dry period, in which the drug concentrations were expected to be higher than under wetter weather conditions. However, very low drug concentrations were measured. It can therefore be assumed that the drugs contained in the water samples pose a very low risk for human health. In order to be able to ascertain whether or not cemeteries are ecologically harmless, further studies involving more measurements under different climatic conditions (i.e. including periods with more intensive precipitation) and a wider range of pharmaceuticals (in particular X-ray contrast agents) will have to be carried out.

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