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Identification and significance of phenazone drugs and their metabolites in ground- and drinking water

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Abstract

Residues of three phenazone-type pharmaceuticals have been identified in routine analyses of groundwater samples from selected areas in the north-western districts of Berlin, Germany. Phenazone, propiphenazone, and dimethylaminophenazone have been detected in some wells at concentrations up to the low μg/l-level. Additionally, three phenazone-type metabolites namely 1-acetyl-1-methyl-2-dimethyl-oxamoyl-2-phenylhydrazide (AMDOPH), 1-acetyl-1-methyl-2-phenylhydrazide, and dimethyloxalamide acid-(*N*′-methyl-*N*-phenyl)-hydrazide have also been identified in these groundwater samples. The residues are suspected to originate from former production spills of a pharmaceutical plant located in a city north of Berlin. It was observed that with the exception of AMDOPH all other residues were efficiently removed during conventional drinking water treatment. The drug metabolite AMDOPH deriving from dimethylaminophenazone residues was found at concentrations of 0.9 μg/l in finished drinking water. However, a following study on the toxicological relevance of the AMDOPH residues has shown that there is no toxicological harm for humans at the low concentrations of AMDOPH observed in Berlin drinking water.

Keywords: Pharmaceuticals; PhACs; Residues; Groundwater; Drinking water; Contamination

1. Introduction

In recent years, the occurrence and fate of pharmaceutically active compounds (PhACs) in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry (Stan and Heberer, 1997; Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; Daughton and Jones-Lepp, 2001; Heberer, 2002a). PhACs are originally designed to sustain the human health. Nevertheless, they may also occur at low concentrations in the aquatic environment. The related hygienic risks are globally limited but specific cases have to be carefully investigated, to prevent any sanitary or hygienic risks.

There are different sources for PhACs in the aquatic environment. One of the major sources is their application in human medical care. The disposal of unused medication via the toilet seems to be of minor importance but many of the pharmaceuticals used are not eliminated in the human body. Often they are excreted only slightly transformed or even unchanged mostly conjugated to polar molecules (e.g. as glucoronides). These conjugates can easily be cleaved during sewage treatment and the original PhACs will then be released into the aquatic environment by discharges of municipal sewage or hospital effluents. Several investigations have shown some evidence that substances of pharmaceutical origin are not completely eliminated during waste water treatment and also not biodegraded in the environment

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(Ternes, 1998; Daughton and Ternes, 1999; Heberer, 2002b). Under recharge conditions, residues of PhACs may also leach into groundwater aquifers. Thus, they have already been reported to occur in ground- and drinking water samples from water treatment plants using bank filtration or artificial groundwater recharge downstream from municipal sewage treatment plants (Heberer et al., 1997; Heberer and Stan, 1997; Heberer, 2002b).

The presence of PhACs from human medical care in groundwater may, however, also be caused by other sources such as manufacturing residues. Nowadays, strong regulations and advanced manufacturing practices shall prevent such spills, especially in the industrialized countries. In the past, regulations were not as strong and in several cases the release of production residues was either tolerated or even accepted. Such spills could result in Superfund sites which may be responsible for today's findings of PhAC residues in groundwater samples. This paper describes the occurrence and identification of such residues in ground- and drinking water samples from a water treatment plant.

2. Experimental

2.1. Materials

All solvents were of HPLC or SupraSolv® purity obtained from Mallinckrodt-Baker, Weinheim, Germany and from Merck, Darmstadt, Germany. Phenazone, dimethylaminophenazone and 1-acetyl-1-methyl-2-phenylhydrazid (AMPH) were obtained from Sigma-Aldrich, Steinheim, Germany. Propiphenazone was purchased from Ferak, Berlin, Germany. 1-acetyl-1-methyl-2-dimethyl-oxamoyl-2-phenylhydrazid (AMDOPH) was synthesized according to a slightly modified method described by Charonnat and Delaby (1930).

2.2. Synthesis of AMDOPH

Thirty gram of dimethylaminophenazone were dissolved in 30 ml deionized water. The solution was cooled in an ice bath and 19 ml of 30% H_2O_2 were added. The colorless solution was extracted three times with 20 ml of ethyl acetate. The organic phases were united and the solvent was removed by drying under a gentle stream of nitrogen. The characterization of the synthesized compound was done by GC/MS.

2.3. Sample preparation

2.3.1. Water samples

The samples were extracted by solid phase extraction (SPE) using an extraction system (Autotrace SPE Workstation) from Tekmar-Dohrmann (Ohio, USA) with cartridges containing 200 mg of Bakerbond styrenedivinylbenzene adsorbent. 10 ng of diphenyl-d_{10} were added as surrogate standard to 500 ml of the water sample and the sample was adjusted to pH 9.0 using a sodium EDTA solution (33 vol% in H_2O). Conditioning of the cartridges was performed applying 2×10 ml of methanol and 2×10 ml of deionized water. The sample was then percolated through the cartridge at a maximum flow rate of approximately 8 ml/min. After drying the cartridge for 60 min by flushing with nitrogen, they were eluted with 10 ml of ethyl acetate for GC analysis and with 10 ml of isopropanol for analysis by HPLC. The eluate was reduced under a gentle stream of nitrogen to a final volume of 500 μl.

2.3.2. Filter sludge samples

The extraction was done by accelerated solvent extraction (ASE). The sludge was obtained by filtrating 10 l of filter flushing water. An aliquot of the sludge sample which was not completely dried to avoid losses of the analytes was extracted using the ASE™ 200 (Dionex Idstein, Germany). In parallel, another aliquot of the sample was used to determine the dry weight of the sludge and to calculate the content of the residues in the dry weight. The temperature for the ASE was set to 150 °C, the pressure was 10.34 MPa and the elution was done with twice 10 ml ethyl acetate. The eluate was reduced to a final volume of 500 μl under a gentle stream of nitrogen.

2.4. GC–MS procedure

The mass spectrometric measurements were performed with an HP5973 MSD combined with an HP6890 gas chromatograph both from Agilent, Waldbronn, Germany. Gas chromatographic separation was performed using a 30 m HP-5MS (5% phenyl methyl siloxane) column with 0.25 mm i.d. $\times$ 0.25 μm film thickness. The injection volume was 1 μl. The temperature program was 1 min at 70 °C, 10 °C/min to 190 °C, 30 °C/min to 300 °C and 5 min at 300 °C. Mass spectrometric measurements were performed using electron impact ionisation (EI) at 70 eV. The mass spectrometer was run in full-scan mode from 40 to 400 amu with a scan rate of four scans per second. The interface temperature was set to 280 °C, the temperature of the MS source was 230 °C.

2.5. HPLC procedure

Liquid chromatographic separation was performed using a XTerra™ RP18 column (Waters, Eschborn, Germany), 5 μm, 3.9×150 mm at 30 °C. Injection volume was 10 μl. The flow was set to 1.0 ml/min, eluent A was water (pH 8.5) and eluent B was acetonitril. The following gradient was used for separation: 30 min 95%

A + 5% B, 0.5 min 70% A + 30% B, 5.0 min 100% B, 5.0 min 95% A + 5% B. Detection was performed using a photodiode detector (996PDA from Waters, Eschborn, Germany). The analytes were identified by their retention times and their corresponding UV spectra (scan range: 193–400 nm). Quantification was performed at a wavelength of 260 nm.

3. Results and discussion

3.1. Phenazone residues in groundwater samples

Three phenazone type pharmaceuticals have been detected in routine analysis of groundwater samples from selected areas in the north-western districts of Berlin, Germany. In these areas, the groundwater aquifers are under the influence of surface water from the Havel river used for groundwater recharge. The compounds that occurred in some of the wells were phenazone, propiphenazone and dimethylaminophenazone. The structures of these compounds are shown in Fig. 1.

The average concentrations of the phenazone-type pharmaceuticals in groundwater from contaminated areas were approximately 3 μg/l for phenazone, 1 μg/l for propiphenazone and 0.4 μg/l for dimethylaminophenazone. In Germany, the use of dimethylaminophenazone was stopped in 1978 because of its potential to form the carcinogenic *N*-nitrosodimethylamine in acidic media (Bresser, 1995). Thus, the recent findings of this substance in groundwater may indicate the existence of residual plumes in the subsoil. It is also possible that the observed concentrations only reflect much higher initial concentrations in the aquifer. Additionally, dimethylaminophenazone was not detected in groundwater under the influence of surface water receiving high loads of municipal sewage effluents in the south of Berlin. The most probable explanation of these findings is a former pharmaceutical-producing plant near Oranienburg, a small town north–west of Berlin, that produced the phenazone-type pharmaceuticals under investigation. Thus, it can be assumed that production residues may have been released into the Havel river or the subsurface where they could occur as bound residues or being dissolved in the aqueous phase.

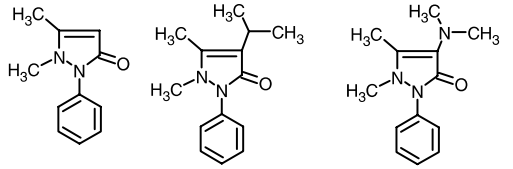

Fig. 1. Structural formulae of the phenazone-type pharmaceuticals.

3.2. Identification of AMDOPH

Three additional peaks were detected in the groundwater samples with mass fragments typical for phenazone-type compounds. Further efforts have been undertaken to identify these compounds applying mass spectrometry.

The dominating phenazone-type compound was identified as AMDOPH with its mass spectrum shown in Fig. 2. There was a distinct peak for this substance even in the total ion chromatogram recorded in full scan mode. A reference substance was not commercially available, therefore the compound had to be synthesized as described in Section 2.2. The mass spectrum and the retention time of the reference substance matched with those of the compound detected in Berlin groundwater.

AMDOPH is described as an oxidation product of dimethylaminophenazone. The formation of AMDOPH from dimethylaminophenazone was observed in the presence of singlet oxygen (Duchstein et al., 1988), sodium periodate (Weber and Wollenberg, 1988) and hydrogen peroxide (Weber and Bresser, 1996). AMDOPH was also found as a product of photochemical decomposition (Marciniec, 1984, 1985a,b). Charonnat and Delaby (1929, 1930) described AMDOPH as a compound with pharmaceutical properties comparable to dimethylaminophenazone. Fig. 3 shows the reconstructed ion chromatograms of the indicative ion traces of AMDOPH analyzed by GC–MS in full scan mode in finished water from a water treatment plant.

3.3. Identification of 1-acetyl-1-methyl-2-phenylhydrazide

The second compound was identified as 1-acetyl-1-methyl-2-phenylhydrazide (AMPH). This compound has been described as secondary product of the oxidation of phenazone derivatives (Marciniec, 1985a; Weber and Bresser, 1996). The mass spectrum of the compound matched the spectrum of the reference substance. The mass spectrum of AMPH is shown in Fig. 4.

3.4. Identification of dimethyloxalamide acid-(*N*′-methyl-*N*-phenyl)-hydrazide (DMOAS)

The mass spectrum of the third compound was very similar to the spectrum of AMDOPH but the fragment with *m*/*z* 191 was missing (Fig. 5). The compound was identified as dimethyloxalamide acid-(*N*′-methyl-*N*-phenyl)-hydrazide (DMOAS). Compared to the other two metabolites this substance was present at trace concentrations only. DMOAS was not yet described as oxidation product of phenazones in the literature but it was observed as a heating product of AMDOPH in sulfuric acid (Beilstein, 1959).

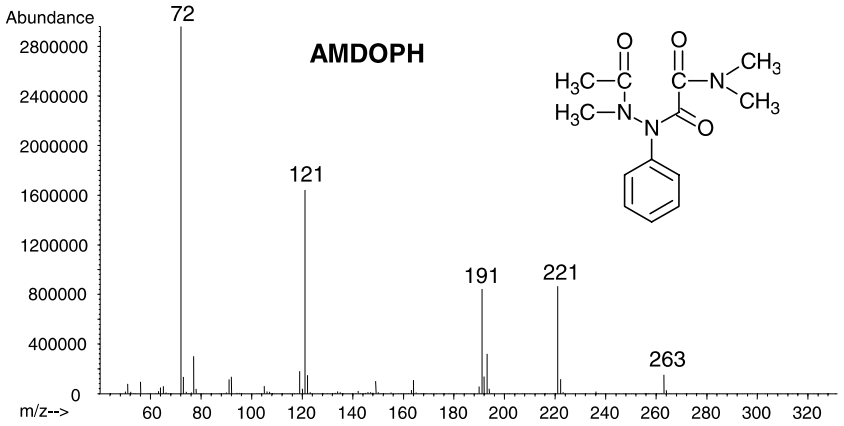

Fig. 2. EI mass spectrum (70 eV) and structural formula of AMDOPH.

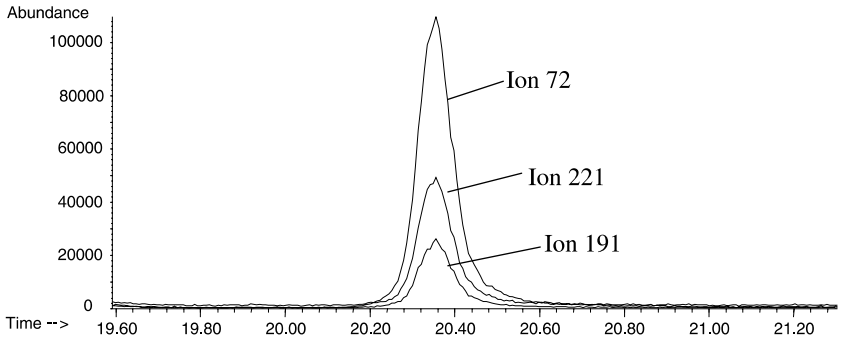

Fig. 3. Reconstructed ion chromatogram of the indicative ion traces of AMDOPH in purified water from a water works analyzed by GC–MS in full scan mode.

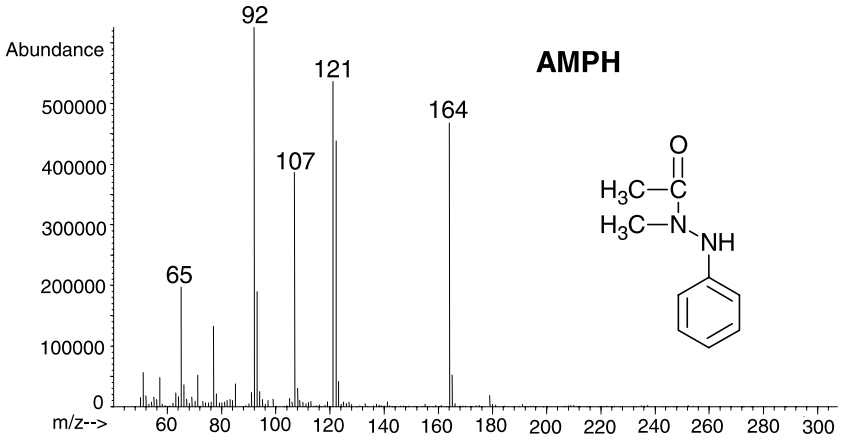

Fig. 4. EI mass spectrum (70 eV) and structural formula of AMPH.

3.5. Attenuation of phenazone residues during drinking water treatment

During drinking water treatment, a significant reduction of the concentrations of the phenazone residues was observed. The treatment consists of aeration of the groundwater followed by multi-media filtration especially to remove iron and manganese. The filter media are composed of expanded clay and sand and were found to be biologically active. Currently, no further

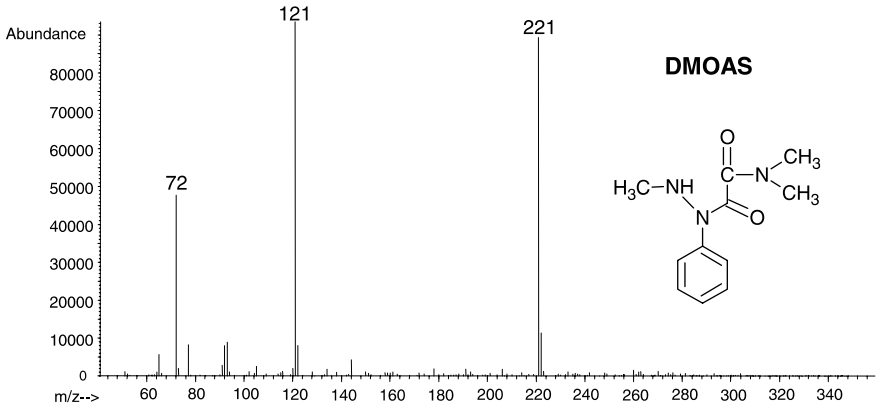

Fig. 5. EI mass spectrum (70 eV) and structural formula of DMOAS.

drinking water treatment such as chemical disinfection is used in the city of Berlin, Germany. A comparison of the concentrations of phenazone residues was done between the raw water (aerated groundwater collected from different waterworks wells) and the filtered water. The raw water and the finished drinking water was sampled twice to investigate the fate of the phenazone residues.

Table 1 shows the results from this study and also includes some previous data measured for phenazone and propiphenazone. About 90% of phenazone and propiphenazone and more than 95% of dimethylaminophenazone were removed during drinking water treatment. However, the removal of AMDOPH (25%) was not as efficient as observed for the other phenazone residues.

The sludge from the backwash water of the filter was analyzed to prove whether the reduction of the concentration of phenazone residues is caused by adsorption to particles or by microbiological processes. In the sludge only AMDOPH was present at concentrations of 0.3 μg/g dry weight whereas the other phenazone-type residues could not be detected. Thus, it was assumed that phenazone, propyphenazone, and dimethylaminophenazone were removed (or degraded to other metabo-lites) during the treatment by microbiological processes. Further studies investigating these processes are in progress.

Additional investigations were also carried out to prove the toxicological relevance of the metabolite AMDOPH being the dominating residue in drinking water. A recent study commissioned by the German Federal Environmental Protection Agency (UBA, 2001) concluded that the occurrence of AMDOPH in drinking water is not desirable but a lifetime consumption of drinking water containing AMDOPH at concentrations observed in Berlin ground- or drinking water will not cause any adverse human health effects. The German Federal Environmental Protection Agency recommends a concentration of 3 μg/l for AMDOPH in drinking water as being tolerable for lifetime consumption (UBA, 2001). The maximum concentrations of AMDOPH in

Table 1
Reduction of phenazone residues during drinking water treatment at a waterworks in Berlin, Germany

Substance	Limit of quantification (ng/l)	Aerated raw water		Drinking water		Removal	
		Ø conc. (ng/l)	RSD (%)	Ø conc. (ng/l)	RSD (%)	Rate (%)	RSD (%)
Phenazone[a]	50	3950	26	400	69	90	7
Propiphenazone[a]	5	1230	29	120	54	90	5
Dimethylamino-phenazone	50	400	–	ND[b]	–	>95	–
AMDOPH	10	1200	–	900	–	25	–
AMPH	20	E 20–100[c]	–	E 30[c]	–	–	–
DMOAS	10	Traces	–	Traces	–	–	–

[a] $N = 6$, for all other compounds $N = 2$.
[b] ND: not detected.
[c] E: Estimated values.

Berlin finished drinking water are clearly below the recommended concentration level.

4. Conclusions

Three phenazone-type pharmaceuticals have been detected in routine analysis of groundwater samples from selected areas in the north-western districts of Berlin, Germany. Additionally, three metabolites of phenazone-type pharmaceuticals have been identified in groundwater. It was shown that except of the metabolite AMDOPH all these residues were efficiently removed during conventional drinking water treatment. A toxicological study proved that there is no harm to humans by life-time consumption of drinking water containing AMDOPH at the maximum concentrations found in Berlin drinking water. The maximum concentrations of AMDOPH in Berlin finished drinking water are clearly below the tolerable concentration level of 3 μg/l recommended by the German Federal Environmental Protection Agency (UBA).

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