EXHIBIT 7

Pentobarbital Found in Ground Water

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Abstract

A landfill in Florida which received wastes in 1968 and 1969 has contaminated nearby shallow ground water. Analysis of a sample of contaminated ground water collected in 1984 from a well approximately 300 m from the landfill tentatively indicated the presence of the addictive sedative pentobarbital, among many other compounds. A well at the same location was resampled in 1991, and pentobarbital was positively identified using gas chromatography/tandem mass spectrometry. The persistence over 21 years of a supposedly unstable drug in anoxic ground water raises the possibility that other pharmacologically active compounds may be found in plumes coming from landfills that have accepted medical wastes.

Introduction

The Hipps Road Landfill was excavated in a swampy area over a Coastal Plain aquifer of 30 m approximate thickness in Jacksonville, Florida. The landfill received wastes from two large naval aviation bases during the period 1968 to late 1969. Although the landfill's permit only allowed it to receive ordinary solid wastes, witnesses and physical evidence show that it also received chemical wastes from aircraft maintenance, which was the major activity at the two bases. A large hospital which was located at one of the naval bases may also have contributed wastes to the landfill. The landfill was placed on the U.S. Environmental Protection Agency's National Priority List for cleanup under the Superfund program in 1983.

The work described in this report was part of an effort to determine the identities of organic compounds in groundwater and drinking-water well samples previously collected from the vicinity of the landfill.

Hydrogeology

The Hipps Road Landfill is located in the southwestern portion of Jacksonville, Duval County, Florida. Figure 1 is a map of the landfill vicinity.

The immediate vicinity of the landfill is relatively flat with elevations between 18.6 and 21.6 m. Farther to the east and north, the land surface slopes down to the Ortega River, whose elevation is less than 6 m, and an unnamed tributary. To the west, there is little relief for a distance of several miles. The landfill vicinity is poorly drained.

The area is underlain at depth by the Floridan Aquifer, whose uppermost unit is the Ocala Limestone of Eocene age. The Ocala Limestone is overlain by the thick Miocene Hawthorn Formation, which has a high clay content and acts as an aquitard. Above the Hawthorn are two units without formal names, which comprise the surficial aquifer. The contamination discussed in this paper is confined to this hydrogeologic unit. The Upper Miocene or Pliocene deposits consist of sand, shell, sandy clay, and limestone. These are overlain by Pleistocene and Holocene deposits of mostly unconsolidated medium- to fine-grained sand, with some thin clay beds and hardpan. The geology, hydraulics, and chemistry of this unit in Duval County are discussed by Leve (1966), Fairchild (1972), Causey and Phelps (1978), an Spechler and Stone (1983).

Investigations at the Hipps Road site have been largely confined to the uppermost 30 m, all of which is included within the surficial aquifer. In this interval, four subunits have been distinguished at the site (CDM, 1986; Golder, 1989). Uppermost is a sandy layer extending approximately 20 m beneath the land surface. Next deepest is a thin, discontinuous semiconfining bed composed of interbedded silty sand, clayey sand, sandy clay, clay, and marl. Beneath this is a limestone bed, which pinches out to the east. Beneath the limestone is another semiconfining bed.

Water-level measurements show that ground water north and east of the landfill flows toward the northeast, presumably to discharge into marshes around the Ortega River and its unnamed tributary. This is consistent with the topography and with the presence of numerous contaminated wells to the northeast of the landfill.

Previous Investigations

An initial investigation was conducted in 1983 after residential wells in the vicinity developed unusual odors and tastes. This investigation showed that the contamination was centered in a plume extending northeastward from the landfill in the lower part of the sand layer (Figure 1). There was also some shallow contamination to the southwest of the landfill. The most severely contaminated monitoring well, designated EMW-3I, was located approximately 300 m east-northeast of the landfill; it was constructed of polyvinylchloride, and was screened between depths of 15.1 and

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18.2 m. The presence of the most severe contamination at this location in 1983, along with confirming hydraulic data, indicates that contaminated ground water moved east-northeastward at a velocity of approximately 15 to 20 m/yr (Ross, 1990).

In the 1983 investigation, water samples (with the exception of two samples from drinking-water wells) were only analyzed for volatile organics. In November 1984, a single sample from well EMW-3I was analyzed by gas chromatography/mass spectrometry for volatile and semi-volatile organic compounds at the U.S. Environmental Protection Agency (USEPA) regional laboratory in Athens, Georgia. Results of this analysis are discussed below. Additional sampling in 1985, 1988, and 1989 produced no results relevant to this paper. Altogether, some 91 monitoring wells and 76 private water-supply wells were sampled in the course of these investigations.

Two of the present authors (BR and WPE) reevaluated the available hydraulic and chemical information regarding the site in 1990-91. As part of this work, well DS-1, of PVC construction and screened from 14.0 to 17.1 m, was installed in the immediate vicinity of well EMW-3I.

The ground water at the location of EMW-3I and DS-1 is highly reducing. A sample collected from DS-1 on July 12, 1990, contained less than 0.05 mg/l each of nitrate and nitrite and 1.2 mg/l of ammonium (as N). Such reducing conditions are common in plumes of contaminated ground water emanating from landfills containing large amounts of organic matter.

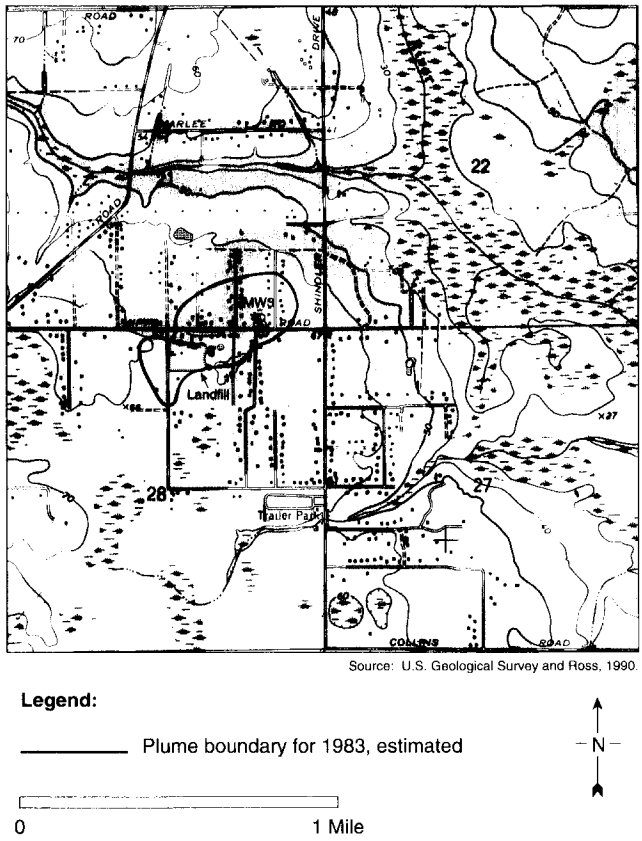

Fig. 1. Hipps Road Landfill NPL site.

Chemical Analysis Methods

Original Sample Analysis. The sample taken on November 1, 1984 from well EMW-3I, designated 85C5431, was analyzed by the USEPA Region IV laboratory on November 26, 1984. The analysis was carried out using gas chromatography/mass spectrometry (GC/MS) with a DB-5 fused silica capillary column. The protocol used was equivalent to USEPA method 625 (extractable organics).

Data Review. The GC/MS data produced by USEPA Region IV were systematically reviewed by interpretation of the mass spectral and gas chromatographic data. The Lee retention index (Lee et al., 1979) was calculated for each unknown compound, and compared to known retention indices from the literature (Rostad and Pereira, 1986; USEPA, 1988). When the mass spectrum of the unknown was deemed to match one of the spectra retrieved by the library search, and when the experimental retention index matched the literature retention index of that library spectrum, a compound was considered to have been identified. Retention indices for several compounds of interest were measured by an outside laboratory. Table 1 gives the rank of the tentative identification (out of the three retrieved mass spectra most closely matching the unknown), the purity (a measure of how closely the unknown and library spectra match, out of a possible score of 1000), and other comments on the identification. In some cases, compounds were identified as structural isomers of the retrieved spectra whose retention index matched that of the unknown.

Gas Chromatography-Tandem Mass Spectrometry Measurements. Selected reaction monitoring gas chromatography/tandem mass spectrometry (GC/MS/MS) experiments to measure pentobarbital were carried out on a Finnigan model TSQ-70B. Samples and spiked standards were prepared by solid phase extraction, followed by evaporation and reconstitution in a minimum volume of methanol. An aliquot of the methanol solution was then analyzed via GC/MS/MS. In this experiment the pseudomolecular ion (M + H, m/z 227) formed by chemical ionization was made to pass through the first mass filter of the instrument. Within the second quadrupole region the molecular ion was allowed to interact with neutral gas molecules, resulting in the formation of daughter ions. Two daughter ions (m/z 97 and m/z 157) determined to be specific for pentobarbital were measured in the third analyzer. Quantitation was performed by a linear correlation to standards of pentobarbital.

Results

The 1984 analysis of the sample from well EMW-3I included comparisons of the mass spectra of unknown contaminants with a library of mass spectra. (Such "library searches" are normally included in analyses by the Superfund Contract Laboratory Program and USEPA regional laboratories). In 1991, these mass spectra were retrieved from storage, and the comparisons were reevaluated.

Twenty-three compounds (Table 1) out of a total of 127 unknowns were identified in the sample by matching both mass spectra and retention indices. Thirty-seven compounds were identified with lower confidence, based on review of the mass spectral library search data only (Table 2). Thirty-four unknowns were characterized partially, and 33 compounds could not be identified with any confidence.

The compounds identified included alkyl benzenes, terpenoids, aromatic organic acids, alkyl phenols, polycyclic aromatics, toluenesulfonamides, phosphate esters, aliphatic organic acids, molecular sulfur, and glycol ethers. Many of these same compounds had previously been identified in landfill leachates (Reinhard et al., 1984). Samples taken from other monitoring wells showed the presence of many of the same compounds as in samples from EMW-3I.

Three compounds of particular interest (in light of the presence of a hospital at one of the two naval air stations) were tentatively identified in the USEPA data by forward mass spectral library searching of mass spectra from the 1984 ground-water sample. These were the hypnotic/sedative pentobarbital, the sedative meprobamate, and the anticonvulsant phensuximide (Windholz, 1989, monographs 7087, 5751, and 7231). These drugs were of interest because the first two are potential drugs of abuse (i.e., they are addictive), and because we considered it unusual to find pharmaceutical compounds in an environmental sample. Moreover, pentobarbital is known to be unstable in solution (Windholz, 1989), so its persistence over 14 years in ground water would not have been predicted.

The spectrum that eventually was positively identified as pentobarbital appeared in the sample at Lee retention index of 295.45. The best three library matches were, in order, pentobarbital, probarbital, and butabarbital. The measured retention index for pentobarbital (293.65), when compared to the experimental retention index from the 1984 sample, indicates that pentobarbital is probably the correct identification.

The identification of meprobamate was also tested by measuring the retention index. The measured value was 301.69, again in good agreement with the retention index of 304.55 for the unknown. The matching score for meprobamate was low (605 out of 1000), but the library spectrum for meprobamate was incomplete (only 19 peaks). If the unknown spectrum had been compared to the complete spectrum of meprobamate, the matching score would undoubtedly have been higher. We did not measure the retention index of phensuximide.

The partial confirmation of the identification of pentobarbital in the ground water at Hipps Road landfill suggested that it might still be present in the ground water seven years later. A sample of water from well DS-1 was collected on August 5, 1991 and was analyzed for pentobarbital by selected reaction monitoring GC/MS/MS, as described above. Pentobarbital was positively identified in the ground-water sample at a concentration of 1 part per billion.

Conclusions

Pentobarbital has been positively identified in a ground-water sample collected at a place and time 300 m and 21 years distant from the apparent source. Thus this compound, which is known to slowly decompose in solution (Windholz, 1989) has persisted for 21 years in an anoxic ground-water environment. Such persistence has not previously been documented for a widely used drug like pentobarbital. The possibility of contamination of ground water by drugs such as pentobarbital and meprobamate should be considered when investigating landfills known to have accepted medical wastes. The fact that some pharmaceuticals degrade readily in an oxic environment (such as normal storage) does not preclude long-term persistence in anoxic ground-water plumes.

Table 1. Compounds Identified at Hipps Road Landfill by Review of Mass Spectral and Retention Index Data

Compound	*Rank/Purity*	*RI:Exp/Ref*[1]
Phenol[2]	1/910	158.08/156.32
1, 4-dichlorobenzene	1/789	164.81/163.15[3]
Indane[2]	3/636	169.87/168.87
2-methylphenol	1/833	173.57/172.86
3-methylphenol[2]	2/901	177.95/177.63
Diethylcarbitol	Manual ID	179.46/174.83[4]
2, 6-dimethylphenol	Isomer of top 3 spectra	184.51/184.08
Isophorone	1/627	187.71/187.34[3]
3-ethylphenol[2]	1/883	196.80/196.96
2, 3-dimethylphenol	1/714	198.65/198.95
Naphthalene	1/595	200.67/200.00
3, 4-dimethylphenol	Isomer of top 3 spectra	201.68/201.49
Benzoic acid[2]	2/799	202.69/200.65[3]
Triglyme	1/727	208.42/205.88[4]
Caprolactam	1/830	215.49/217.00[4]
Skatole	Isomer of best match	237.88/237.80
Diethylphthalate	1/935	271.04/272.56[3]
2-(methylthio) benzothiazole	1/677	273.74/272.73[3]
Pentobarbital	1/766	295.45/293.65[4]
Meprobamate	1/605	304.55/301.69[4]
o-terphenyl	Mass 230 present	316.63/317.43
Di-n-butyl phthalate	Manual ID	326.93/328.00[3]
Bis(2-ethylhexyl) phthalate	1/867	406.14/406.15[3]

[1] Experimental and Reference Retention Index (RI). Reference RI data from Rostad and Pereira (1986) unless otherwise specified.
[2] Compounds also found in landfill leachates by Reinhard et al. (1984).
[3] RI data from USEPA (1988).
[4] RI data from this work.

Table 2. Compounds Identified at Hipps Road Landfill by Review of Mass Spectral Data Only

Compound	*Rank/Purity*	*Exp. RI*	*Comments*
Trimethylbenzene[1]	1, 2, 3/817	160.77	1, 3, 5-isomer purity
Isocineole	1/717	165.32	All 3 spectra $C_{10}H_{18}O$
Diethylbenzene	1, 2, 3/830	167.17	1, 4-isomer purity
Cineole[1]	1/785	169.02	All 3 spectra $C_{10}H_{18}O$
Acetophenone	1/765	176.26	
Fenchone[1]	1, 2/794	181.31	All 3 spectra $C_{10}H_{16}O$
C4-benzene[1]	1, 2, 3/584	186.36	1, 2, 3, 4-tetramethyl-isomer purity
3-caranol	1, 2/424	186.70	
Ethyldimethylbenzene	1, 2, 3/707	187.37	2-/1, 4-isomer purity
Dihydroalphaterpineol	1/715	195.96	
Methylindane[1]	2, 3/768	193.77	5-isomer purity
p-menthan-4-ol[1]	1, 2/853	194.61	
p-menthan-3-ol[1]	1, 2/836	198.32	
3-pinanone	1/720	205.22	Top 2 spectra $C_{10}H_{16}O$
C3-phenol	1, 2, 3/487	214.72	Coelution; 3-ethyl-5-methyl-isomer purity
Benzeneacetic acid	1, 2/847	216.16	
Indanone	1/815	218.86	
C4-phenol	2/467	220.54	Coelution
Benzene-propanoic acid[1]	1/917	230.64	
Alpha-ethyl-benzeneacetic acid	1/617	236.36	
Benzenebutanoic acid	1/775	244.61	
Dihydromethylindole	1/723	252.19	
Phenylphenol	1, 2/791	259.60	
Dimethylbenzenebutanoic acid	1/846	266.33	2, 5 isomer
N, N-diethyl-3-methyl benzamide	1/843	269.36	"DEET"
C8-benzene	1, 2, 3/484	275.59	1, 5-dimethyl-2, 4-bis(isopropyl) isomer purity
Toluenesulfonamide	1, 3/888	277.10	Ortho isomer purity
Tributylphosphate[1]	1/664	278.79	
C3-benzene-sulfonamide	1, 2/622	281.31	N, N, 4-trimethyl-isomer purity
Phensuximide	1/840	284.01	
N-ethyl-p-toluene-sulfonamide	1/794	289.90	
2-chloroethanol, phosphate (3:1)	1/840	295.96	
3-(2-phenylethyl) phenol	1/625	307.33	Second-best match is 4-(2- isomer
Hexadecanoic acid[1]	1/644	325.15	
Molecular sulfur (S8)	1/907	343.96	
N-cyclohexyl-p-toluene sulfonamide	1/702	356.63	
Bisphenol A	1/756	359.41	

[1] Compounds also found in landfill leachate by Reinhard et al. (1984).

Acknowledgments

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