In recent years, there has been growing interest in the occurrence, fate, and possible effects of human and veterinary drug residues in the environment (Daughton and Ternes 1999; Halling-Sørensen et al. 1998; Kümmeter 2001; Witte 1998). Studies with a special focus on drugs used in human medicine have established that these compounds mainly reach surface waters via the release of effluent from sewage treatment plants. Today, up to 80 compounds have been identified and quantified in the low range of nanograms to micrograms per liter (Heberer 2002). Studies performed in the United Kingdom, Denmark, Germany, and the United States reveal that these agents represent a new class of organic environmental contaminants worldwide (Kümmeter 2001). There is concern about effects resulting from the entry of these compounds into the environment, including the possibility of the spread of antibiotic resistance (Witte 1998) and/or effects on the endocrine system because of the ability of some of these compounds to behave as hormones (Daughton and Ternes 1999).

At present there are very few established routes for the entry of veterinary drugs into the environment. Recently, sophisticated analytical liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) has led to the detection of tetracyclines on farmed land at concentrations of up to 300 µg/kg soil, which demonstrated that this group of antibiotics is persistent and can accumulate in soil after repeated fertilization with liquid manure from intensive pig farming. Furthermore, field studies gave no proof of leaching of these compounds into deeper soil segments or into groundwater because of the strong sorption of the drugs in topsoil (Hamscher et al. 2000, 2002). Presently, there is only limited information available on the direct effects of these drugs on soil biota. Investigations in this field are difficult to perform because the soil microorganism community is a very complex system with at least 90% of the bacteria living in this compartment unidentified (Nwosu 2001).

Large-scale use of tetracyclines and several other veterinary drugs (e.g., various sulfonamides, tylosin) in pig production is common not only within the European Union (Anonymous 2001) but also in the United States (Kolpin et al. 2002) and, to our best knowledge, in China, Southeast Asia, and Russia. These drugs are in use or have been in use for many years as feed additives and for prophylactic, metaphylactic, and therapeutic purposes.

Large-scale pig production represents a considerable source of dust (Hartung 1997, 1998; Pedersen et al. 2000). This results both in high dust exposure for farmers and farm workers in animal confinement buildings, causing respiratory health hazards (Iversen et al. 2000; Nowak 1998; Platz et al. 1995; Radon et al. 2002), and in emissions of dust particles into the environment by way of the exhaust ventilation air (Hartung 1995; Seedorf and Hartung 2002). About 85% of the dust from animal confinement buildings consists of organic material, including protein (from pig skin), animal feed, endotoxins, fungi, and bacteria (concentrations of up to 50 million colony-forming units per gram of dust) (Hartung 1997). Today, there is no doubt regarding the health hazards of dust in animal confinement buildings, but there is still little knowledge concerning the possible risk of specific substances in dust (Nowak 1998). To determine whether antibiotics may also be contaminants in dust from animal confinement buildings, we undertook a retrospective study to analyze dust samples collected from a pig-fattening farm during the years 1981 to 2000 for the occurrence of various antibiotics, including tetracyclines, sulfonamides, tylosin, and chloramphenicol.

Materials and Methods

Collection of dust samples. We studied sedimentation dust collected from 1981 to 2000 in a 350–420-head pig finishing unit (60–110 kg live weight) over periods of 14–30 days using a standardized metal frame with an effective sampling surface area of 3,002 cm² (38 × 79 cm) covered with fresh aluminum foil. The sampling frame stood approximately in the middle of the pig house, where there was no exposure to high air currents, 1.5 m above the floor, which is the typical breathing height of humans. After the collecting period (each year 10–15 samples were collected in the piggery, one of which was then randomly selected for analysis in this study), technicians carefully sampled the dust from the aluminum foil using a clean, new brush and placed it into glass vials sealed with tight stoppers. Before sampling, we removed any remaining dead insects, spiders, and coarse particulate matter originating from ceiling materials. After the collection process, technicians covered the metal frame with fresh aluminum foil for the next collecting period and removed the glass containers to the laboratory, where they were allowed to cool down gradually for storage at 4°C.

Sample preparation and measurement. We removed 0.1 g samples from each of the glass...
containers and mixed them with 1.0 mL citrate buffer (pH 4.7), twice-extracted with 6 mL ethyl acetate as previously described for soil and liquid manure (Hamscher et al. 2002). We evaporated ethyl acetate to dryness and reconstituted samples with 1 mL 90% acetonitrile/10% 100 mM ammonium acetate.

We conducted high-performance liquid chromatography (HPLC) separation on a Purosil C18 Column (Waters Corp., Milford, MA, USA) with a gradient solvent system consisting of 0.5% formic acid (Riedel-de Haen, Seelze, Germany) in water containing 1 mM ammonium acetate (Merck, Darmstadt, Germany) (solvent A, pH 2.5) and acetonitrile (Baker, Griesheim, Germany) (solvent B), using an injection volume of 8 μL. We measured all compounds under investigation using two HPLC runs. First, we used the conditions recently described for the separation of tetracyclines, tylosin, and chloramphenicol (Hamscher et al. 2002). We baseline separated and analyzed seven sulfonamides with a modified gradient system for the second run (i.e., 100% solvent A for 1 min, linear gradient to 25% solvent B for 9 min, linear gradient to 50% solvent B for 1 min, and finally 50% solvent B for 3 min). After elution of the antibiotics, we rinsed the column for 3 min with 99% solvent B and reequilibrated it with 100% solvent A for 8 min.

We performed tandem mass spectrometry (MS-MS) for detection using an LCQ ion trap with an electrospray ionization source (Finnigan Mat, San Jose, CA, USA), with the trap with an electrospray ionization source (Finnigan Mat, San Jose, CA, USA), with the spray needle voltage was set to +5 kV for chloramphenicol and +5 kV for all other compounds. In the case of chloramphenicol, we turned the source fragmentation –5 kV for chloramphenicol and +5 kV for all other compounds. In the case of chloramphenicol, we turned the source fragmentation on with a collision energy set at 10 V. Drying gas was nitrogen generated from pressurized air in an Ecoinert 2 ESP nitrogen generator (DWT-GmbH, Gelsenkirchen, Germany).

We set the sheath gas flow at 100 units and mixed them with 1.0 mL citrate buffer (pH 4.7), twice-extracted with 6 mL ethyl acetate as previously described for soil and liquid manure (Hamscher et al. 2002). We evaporated ethyl acetate to dryness and reconstituted samples with 1 mL 90% acetonitrile/10% 100 mM ammonium acetate.

We conducted recovery studies with residue-free dust samples at concentrations of 0.2, 0.5, and 1.0 mg/kg. We calculated the recovery rates as an average of three individual experiments. The limit of quantification based on these studies was 0.1 mg/kg for the tetracyclines and tylosin and 0.05 mg/kg for the sulfonamides. The limit of detection was approximately 2-fold lower.

### Results

Table 2 shows the summary of all results for this retrospective study, and Figure 1 presents the molecular structures of all detected antibiotics. We detected up to five different antibiotics at total concentrations ranging from 0.2 to 12.5 mg/kg dust in 18 of 20 samples; chromatograms and mass spectra of a sample containing five antibiotics are shown in Figure 2. Tylosin was present in 16 of 20 samples, three of which had concentrations of > 5 mg/kg. In 13 samples, sulfamethazine was present at concentrations of up to 2.9 mg/kg, and several tetracyclines were present in 12 samples (0.2–5.2 mg/kg). In another three samples, we detected chloramphenicol—which

### Table 1. Characteristics of HPLC and MS-MS methods: retention times (RT), optimized MS-MS parameters, and product ions for the determination and quantification of various antibiotics in dust.

| Method/compound | RT (min) | Precursor mass (m/z) | Collision energy (%) | Product ions, m/z (relative abundance, %) |
|-----------------|---------|---------------------|---------------------|------------------------------------------|
| HPLC method 1   |         |                     |                     |                                          |
| Oxytetracycline | 7.21    | 461                 | 20                  | 426 (7), 443 (100), 444 (9)               |
| 4-epi-Tetracycline | 7.17 | 445                 | 20                  | 410 (6), 427 (100), 428 (13)              |
| Tetracycline    | 7.52    | 445                 | 20                  | 410 (4), 427 (100), 428 (7)              |
| 4-epi-Chlortetracycline | 8.07 | 479                 | 27                  | 444 (69), 461 (51), 462 (100)            |
| Chlortetracycline | 8.48 | 479                 | 27                  | 444 (51), 461 (54), 462 (100)            |
| Tylosin         | 9.60    | 917                 | 29                  | 754 (3), 772 (100)                       |
| Chloramphenicol | 10.34   | 321                 | 24                  | 176 (9), 194 (100), 237 (8), 249 (13), 257 (10) |

*Abbreviations: —, not detectable; CAP, chloramphenicol; CTC, chlortetracycline; OTC, oxytetracycline; SMZ, sulfamethazine; TC, tetracycline; TYL, tylosin. The values (mg/kg dust) represent the means of two replicates per sample, which have been corrected for mean recovery investigated in the concentration range of 0.2–1.0 mg/kg: 103 ± 21% for OTC, 89 ± 21% for TC, 94 ± 18% for TYL, 27 ± 8% for TYL, and 49 ± 18% for SMZ. Calculations for CAP were based on the method of standard addition as described in “Materials and Methods.” SMZ was the only sulfonamide that could be detected. Including their 4-epimers.

### Table 2. Antibiotic residues in pig-house dust.

| Sampling year | OTC (mg/kg) | TC* (mg/kg) | CTC* (mg/kg) | TYL (mg/kg) | CAP (mg/kg) | SMZ (mg/kg) | Sum (mg/kg) |
|---------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|
| 1981          | 1.10        | —           | —            | 0.42        | —           | 1.85        | 3.37        |
| 1982          | 0.18        | —           | —            | 0.09        | —           | 0.06        | 0.33        |
| 1983          | —           | 0.19        | 2.12         | 5.65        | —           | 2.90        | 10.66       |
| 1984          | —           | —           | —            | —           | —           | —           | —           |
| 1985          | —           | —           | —            | —           | —           | —           | —           |
| 1986          | —           | —           | —            | 12.18       | —           | 0.32        | 12.50       |
| 1987          | —           | —           | —            | 8.72        | —           | 0.39        | 9.11        |
| 1988          | —           | —           | —            | 0.72        | —           | 0.43        | 1.15        |
| 1989          | —           | —           | —            | 0.45        | 1.96        | 0.34        | 2.75        |
| 1990          | —           | —           | —            | 0.14        | —           | 0.09        | 0.23        |
| 1991          | 0.43        | —           | 0.32         | 0.26        | 9.07        | 0.41        | 10.49       |
| 1992          | —           | —           | —            | 0.35        | 5.49        | 0.05        | 5.89        |
| 1993          | —           | —           | —            | 0.19        | —           | 0.12        | 0.41        |
| 1994          | —           | —           | —            | 0.23        | —           | 0.12        | 0.37        |
| 1995          | —           | 0.37        | 0.52         | 0.29        | —           | —           | 0.18        |
| 1996          | 0.29        | 5.18        | —            | 0.55        | —           | 0.16        | 6.18        |
| 1997          | —           | 0.47        | 0.16         | —           | —           | —           | 0.63        |
| 1998          | —           | 0.50        | 0.20         | —           | —           | —           | 0.70        |
| 1999          | —           | 0.61        | —            | —           | —           | —           | 0.61        |
| 2000          | —           | 0.19        | —            | —           | —           | —           | 0.19        |

*Abbreviations: —, not detectable; CAP, chloramphenicol; OTC, chlortetracycline; OTC, oxytetracycline; SMZ, sulfamethazine; TC, tetracycline; TYL, tylosin. The values (mg/kg dust) represent the means of two replicates per sample, which have been corrected for mean recovery investigated in the concentration range of 0.2–1.0 mg/kg: 103 ± 21% for OTC, 94 ± 18% for TC, 49 ± 18% for TYL, 27 ± 8% for TYL, and 49 ± 18% for SMZ. Calculations for CAP were based on the method of standard addition as described in “Materials and Methods.” SMZ was the only sulfonamide that could be detected. Including their 4-epimers.
has been prohibited for use in animal husbandry in the European Union since 1994—at levels between 2.0 and 9.1 mg/kg.

**Discussion**

The use of high amounts of veterinary drugs has led to the occurrence of tetracycline and sulfonamide residues in liquid manure and soil, as well as in surface water and, in the case of sulfonamides, also in groundwater (Berger et al. 1986; Hamscher et al. 2000, 2002; Langhammer et al. 1988; Lindsey et al. 2001; Winckler and Grafe 2001). The highest concentrations occurred in liquid manure (milligram per kilogram range) and in soil (microgram per kilogram range), with trace amounts in surface water and groundwater samples (lower microgram per liter range). In comparison, the present investigation showed that dust originating from a pig-fattening farm represents a new route of entry into the environment for drugs applied in animal houses. The lower milligram per kilogram concentration range and the number and frequency of compounds detected in dust may indicate a possible health risk for humans via this environmental source. The antibiotics in dust may originate mainly from animal feed mixed with veterinary drugs, for example, for therapeutic use. This feed is usually in powder or pellet form, which can release distinct amounts of dust during handling. Another source of antibiotics may be dried liquid manure particles, which are regular constituents of dust in animal confinement buildings (Donham 1993). Because sulfonamides and tetracyclines are poorly metabolized in pigs, high amounts of the parent drugs are therefore excreted, and these substances build residues in liquid manure (Berger et al. 1986; Donham 1993; Hamscher et al. 2002; Winckler and Grafe 2001). We recently demonstrated the stability and accumulation of tetracyclines in dried liquid manure particles in environmental samples (Hamscher et al. 2002).

High dust exposure in animal confinement buildings may be a respiratory health hazard mainly because of the high contents of antibiotics. A recent survey on dust in pig-fattening buildings in Europe revealed average concentrations of inhalable airborne dust of 2.2 mg/m³ (Takah et al. 1998). Consequently, a farmer working 8 hr/day in a confined pig building inhales about 6.3 mg of dust contaminated with approximately 0.02 µg of various antibiotics, assuming an average tidal volume of 0.5 L. 12 breaths/minute under resting conditions, and a total concentration of 3.4 mg of antibiotics per kilogram of dust (mean value derived from Table 2). This example includes several variables and can only give an estimate of the amount of antibiotics entering the respiratory tract of humans. In practice, the concentration of the antibiotics in the dust can be three times higher than that used in the calculation (Table 2). Furthermore, the dust concentration in the air is usually higher in winter than in summer, and the breathing rate can also be distinctly higher during work (up to 45 L), resulting in distinctly higher inhaled amounts of inhaled dust and antibiotics.

Although the resulting local concentration of antibiotics in the lung is far too low for any bacteriocidal or bacteriostatic effect, permanent exposure to subtherapeutic concentrations of various antibiotics represents optimal conditions for the development of antibiotic resistance.

An additional conclusion drawn from our study concerns the issue of dust as a source that can provide enormous amounts of information about the former and present use of veterinary drugs in intense livestock production. In the early 1980s, there was heavy use of tylosin in pig production, which is reflected in the analytical data obtained for 1983 and 1986–1987. Following the growing knowledge of possible allergic health hazards related to this compound (Barbera and de la Cuadra 1989;
Caraffini et al. 1994; Danese et al. 1994; Hjorth and Roed-Petersen 1980) and its ultimate ban as a feed additive in the European Union in 1998, tylosin was no longer detectable in the dust samples collected in 1999 and 2000. We found chlortetracycline in only three samples before its ban in 1994 in intensive livestock farming in the European Union. Farm records reveal reconstruction of the confinement building in 1984; subsequently, no further use of antibiotics was necessary as a result of the animals’ health status. Accordingly, the results show that the dust samples were free of any antibiotic compound for 1984 and 1985. Unfortunately, this antibiotic-free period was not permanent, and the data for 1986 show antibiotic use on an even greater scale than in previous years.

Conclusions

A new entrance route for veterinary drugs into the environment has been discovered. We detected substantial quantities of several antibiotics in dust from a pig finishing unit. Further efforts should be undertaken to confirm these preliminary findings, including the investigation of dust from larger pig production systems and from henhouses, and with a higher sampling frequency.

Because there may be adverse effects on animal and human health resulting from the exposure to dust contaminated with antibiotics, future research should take this type of exposure into consideration when assessing health risks to persons exposed to farm dust. This should include monitoring of human health, including the state of antibiotic resistance in farmers to antibiotics they are frequently exposed to.

In order to minimize the possible risks of antibiotics in dust, the use of antibiotics in livestock farming should be reduced whenever possible.

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