Emerging Topics in Ecotoxicology

Laurence S. Shore
Amy Pruden
Editors

Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations

Transport in Water and Soil

Springer

Emerging Topics in Ecotoxicology

For other titles published in this series, go to
www.springer.com/series/7360

Laurence S. Shore • Amy Pruden
Editors

Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations

Transport in Water and Soil

Springer

Editors
Laurence S. Shore
Departments of Endocrinology
and Toxicology
Kimron Veterinary Institute
Bet Dagan
50250 Israel

Amy Pruden
Via Department of Civil
and Environmental Engineering
Virginia Polytechnic Institute
and State University
Blacksburg, VA 24061
USA

ISSN 1868-1344 e-ISSN 1868-1352
ISBN 978-0-387-92833-3 e-ISBN 978-0-387-92834-0
DOI 10.1007/978-0-387-92834-0
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009927503

Cover illustration: Hercules cleaning the stables of Augeus. The stables of Augeus were a CAFO of three thousand cattle. The manure was allowed to accumulate for thirty years and not used for fertilization. Hercules was assigned the task of cleaning the stables in one day. He did this by diverting two rivers to pass through the stables which resulted in the surrounding fields receiving the necessary nutrients. Photo © Maicar Forlag—www.maicar.com.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Steroidal hormones have existed nearly as long as early multi-cellular life forms first emerged. This means that nature has long developed methods for removing them from the environment but it also means that many life forms as simple as corals and snails use the steroid hormones in their reproductive cycles. What is of concern is that the concentrations of steroid hormones released from CAFOs has resulted in concentrations of hormones not previously seen in nature and has the potential to disrupt ecology of the exposed areas. For example, a dairy barn of 100 cows produces about 2 kg of estrogen/year while the estrogens can affect fish and plants at the 10 ng/L level.

Measuring the transport of steroids in the environment has the advantage in that the kg amounts of steroids produced per year by just a small CAFO can be easily traced as they move through the environment. Each of the major steroids produced has different mobility (mobile, partially mobile, and immobile) in the environment and these differences in mobility can be used to characterize the movement of the veterinary pharmaceuticals released from the same source. This is of import as neither the pharmaceuticals nor the steroids follow the physiochemical models that work for pesticides and herbicides. Furthermore, it is difficult to evaluate potential EDCs until we have some understanding of the role of the physiologically active steroids constantly secreted into the environment.

The plethora of pharmaceuticals used in animal husbandry makes it difficult to determine what the most likely compounds to cause risk are. Most of the compounds are released in concentrations several orders lower than their lowest observable effect. However, even very small amount of sulphonamides and tetracycline reaching the soil induce antibiotic resistant genes (ARGs) in soil bacteria, thus the study of ARGs is a new way of evaluating the affects of antibiotics in the environment.

Although in general, the same drugs are used by humans as for animal husbandry, some compounds are of concern because of the particular type of CAFOs in which they are applied. In poultry, the coccidiostats, generally calcium ionophores, have very high toxicity and a large number of physiological effects. The transport patterns and environmental effects of agents used in aquaculture like malachite green and the hormones used to modify sex ratios like norethisterone and methyltestosterone have barely been defined. In the cattle industry, large use is made of the acaricides, avermectins, and the cypermethrins for parasite control. Juvenile growth

hormone inhibitors for fly control are also widely used in cattle, which could affect soil fauna in very small quantities as they reach the environment without any modification. To measure the effects of these acaricides and insecticides, the science of soil ecology needs to be utilized. This field has had some major methodological breakthroughs recently but the ability to evaluate the data still faces major difficulties. Evidence that the steroids themselves can play a role in soil ecology, e.g., estrogens increase the vegetative growth of alfalfa and change the bacterial diversity, suggests new routes of impact for endocrine disruptors.
This book addresses three main topics:

1. The steroidal hormones: What happens to the tons of these physiologically active compounds released into the environment? Can the recent extensive work on the transport of these compounds in soil be used as a model for anthropogenic compounds coming from the same source? Can the hormonal profile be used to determine the source of pollution?
2. Soil ecology: What is utility of studying soil ecology? Which organisms are most likely to be affected? What is the significance of antibiotic resistant genes in bacteria?
3. Pharmaceuticals used in animal husbandry: Which compounds unique to animal husbandry are most likely to have adverse environmental effects?

On a personal note, for the last 30 years, I have been on the trail of the steroid hormones in the environment ever since I observed infertility in cattle ingesting alfalfa with high phytoestrogen content. The high phytoestrogen content was only observed in fields irrigated with treated sewage water. The agents in the sewage water responsible for the high phytoestrogen were estrone and estradiol. This started a search for determining the steroidal hormone concentrations and environmental effects in manures, watersheds, sewage effluent, rivers, topsoil and deep vadose zone. I hope the readers will appreciate this long journey as much as I have enjoyed it. Finally I would like to thank my dear wife, Maxine, for her support which included collecting samples from streams under very adverse conditions.

Bet Dagan, Israel Laurence Shore

Contents

Contributors

Francis X.M. Casey North Dakota State University, 123 Walster Hall, Fargo, ND 58105, USA, francis.casey@ndsu.edu

Thomas Heberer Oldenburg Food Institute, Lower Saxony Federal State Office of Consumer Protection and Food Safety, Martin-Niemöller-Straße 2, 26133 Oldenburg, Germany, Thomas.heberer@laves.niedersachsen.de

Amy Pruden Via Department of Civil & Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24061, USA, apruden@vt.edu

Laurence S. Shore Departments of Endocrinology and Toxicology, Kimron Veterinary Institute, POB 12, Bet Dagan 50250, Israel, shorel@int.gov.il

Yosef Steinberger The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel, steinby@mail.biu.ac.il

Robert L. Wershaw US Geological Survey, Denver Federal Center, Box 25046, MS 408, Denver, CO 80225-0046, USA, rwershaw@usgs.gov

Abbreviations

A	Androstenedione
AOB	Autotrophic ammonia-oxidizing bacteria
APEO	Alkyl phenol ethoxylates
AR	Androgen receptor
ARA	Antibiotic resistance assay
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant genes
As(III)	Arsenide
As(V)	Arsenate
AU	Animal unit
CV	Crystal violet
DHEA	Dihydroepiandrosterone
E1	Estrone
E2	17β-Estradiol
E3	Estriol
EE	Ethinylestradiol
ERE	Estrogen responsive elements
IGF; IGR	Insect growth inhibitors; regulators
K_d	Linear partitioning coefficient
LC50; LD50	Lethal concentration 50%; dose 50%
LCG	Leucocrystal violet
LMG	Leuco-malachite green
LOEC; LOEL	Lowest observable effective concentration; level
Log K_{ow}	Log_{10} octanol–water partitioning coefficient
MGA	Melengestrol acetate
MOE	Margin of exposure
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSP	Manure storage pond
MST	Microbial source tracking
MT	Methyltestosterone
mtDNA	Mitochondrial DNA
NOEC, NOEL	No observable effect concentration; level
P4	Progesterone

P5	Pregnenolone
PCPPs	Pharmaceuticals and personal care products
PLFAs	Phospholipid ester-linked fatty acids
qCO_2	Respiratory quotient
RASFF	Rapid Alert System for Food and Feed
SIR	Substrate induced respiration
SPE	Solid phase extraction
SREF	Streptogramin-resistant *E. faecium*
SWTP	Sewage water treatment plant
T	Testosterone
TbA	Trenbolone acetate
TbOHa; TbOHb	Trenbolone-17α; trenbolone-17β
VRE	Vancomycin-resistant *Enterococci*
Vtg	Vitellogenin
3β-HSD	Δ5,3β-Hydroxysteroid dehydrogenase
17β-HSD	17β-Hydroxysteroid dehydrogenase

Organizations

CDC	Center for Disease Control (US)
CFR	Code of Federal Regulations (US)
EC	European Communities
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency (US)
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
HSUS	Human Society of the United States
LAVES	Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit
PAN	Pestizid Aktions-Netzwerk
USDA	US Department of Agriculture
USDA-ERS	USDA Economic Research Service
USDA-NASS	USDA National Agricultural Statistics Service
US-FDA	US Food and Drug Administration
US-FDA-CVM	US-FDA Center for Veterinary Medicine
USGS	US Geological Survey
WHO	World Health Organization

Chapter 1
Introduction

Laurence Shore and Amy Pruden

Abstract Concentrated animal feeding operations as presently known began after the Second World War. Although mostly developed in the US, with the rise of globalization, the effects of concentration of animal husbandry in small areas can be seen worldwide. In recent years, the rise of aquaculture on a massive scale has presented many new problems that were largely unanticipated.

1.1 Definition of CAFOs

Concentrated animal feeding operation (CAFO) is a regulatory concept for large animal feeding operations, which take advantage of cost effectiveness of large facilities. The same largeness of scale also has disadvantages as it increases point source pollution (Steinfeld et al. 2006). The US government (EPA 2007) defines CAFOs as *agricultural operations where animals are kept and raised in confined situations*. CAFOs congregate animals, feed, feces and urine, dead animals, and production operations on a small land area. Feed is brought to the animals rather than the animals grazing or otherwise seeking feed in pastures, fields, or on range-land (Table 1.1). Alternatively, livestock can be defined in animal units = 1,000 lb (450 kg) live animal wt. and CAFOs are those with more than 1,000 AU or those with 301–1,000 AU with a concentration greater than 2 AU for each acre available for spreading manure.

The European Union terms these facilities as intensive livestock production systems and regulation is based on the manure generated and the land available for application (170 kg/ha/year) (Oenema 2004).

Although aquaculture (Chap. 12) is usually regulated by different protocols than the above CAFO regulations, for environmental purposes, pharmaceuticals used in

L. Shore (✉) and A. Pruden
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute,
POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_1,

Table 1.1 CAFOs as defined by the US Environmental Protection Agency. Poultry facilities are defined by their use of liquid manure handling systems (LM)

Animal sector	Large CAFOs	Medium CAFOs
Cattle or cow/calf pairs	≥1,000	300–999
Mature dairy cattle	≥700	200–699
Veal calves	≥1,000	300–999
Swine (>25 kg)	≥2,500	750–2,499
Swine (<25 kg)	≥10,000	3,000–9,999
Horses	≥500	150–499
Sheep or lambs	≥10,000	3,000–9,999
Turkeys	≥55,000	16,500–54,999
Laying hens or broilers (+LM)	≥30,000	9,000–29,999
Chickens other than laying hens (–LM)	≥125,000	37,500–124,999
Laying hens (+LM)	≥82,000	25,000–81,999
Ducks (–LM)	≥30,000	10,000–29,999
Ducks (+LM)	≥5,000	1,500–4,999

concentrated aquaculture can have major environmental impact and should be considered to be CAFOs. This is especially the case considering that many of the compounds used are the same as those at other CAFOs and reach the environment directly as the parent compound.

1.2 History of CAFOs

The earliest CAFO with widespread environmental damage could have been the Augean's stables with the effluent from the large number of cows making the surrounding areas unlivable (1250 BCE). Modern CAFOs became popular in the decades following WWII. For example, according to the Kansas Board of Agriculture statistics, the percentage of cattle on feed in large Kansas feedlots (1,000 head capacity or more) went from 26.7% in 1960 to 87.6% in 1975 (Wood 1980). During the 1950s, many European countries followed the US lead, but the move to CAFOs in Europe has accelerated in the past decade due to inclusion of large agricultural countries like Poland. Although European CAFOs are generally much smaller than in those in the US, these differences are diminishing, e.g. the same company runs a swine facility with more than 800,000 hogs in both Poland and Utah. Israeli agriculture due to its lack of natural resources and socialist philosophy has been based on the CAFO concept since its inception. In Africa, CAFOs are seen as protecting the environment since sustenance farming there is considered to damage the environment. On the other hand, attitudes towards CAFOs in Asia are generally ambivalent as large scale farming is considered to damage the environment more than the small farm holdings.

1.3 Micropollutants

Until recently, the major emphasis on effluents from CAFOs has been on regulating the major inorganic compounds, nitrates and heavy metals. In the past decade, with the realization that pharmaceuticals are reaching the environment even after treatment in a sewage water treatment plant (SWTP), attention has turned to the pollutants released from CAFOs that could have an effect on the environment in μg/L or even ng/L quantities. The impact of micropollutants from CAFOs is of particular concern since the manure reaches the environment without significant treatment or dilution, which characterizes sewage effluent. Quantitatively, the major physiologically active compounds released are the steroid hormones. Although steroid hormones have been in the environment probably since the first multicellular organism emerged, CAFOs release these hormones in quantities not previously seen in nature until modern times (Table 1.2).

The second category of concern are the antibiotics, which, although they reach the soil in small quantities, affect the emergence of antibiotic resistance bacteria in the soil. Although antibiotic resistance genes imparting the ability to resist antibiotics have probably been around since the first soil microorganisms produced antibiotics, again, the amounts reaching the environment are unprecedented. Also, the compounds from the CAFO are directly applied to soils that may have never been exposed to this form of antibiotic. The major use of antibiotics (chlor- and oxytetracycline, tylosin, bambermycins, virginamycin) in the US is as growth promoters, while this use of antibiotics for this purpose is banned in the EU and Israel. For treating animal diseases, penicillin, tetracycline, streptomycin and sulfonamides are commonly used. In particular, sulfonamides are widely used in the US swine industry. The actual amount and type of antibiotic used varies tremendously between various regions of the US (Table 9.1).

The third category of concern includes other pharmaceuticals used in CAFOs (Table 1.3). Most of these pharmaceuticals are the same as those used by humans and the available information on these compounds has recently been extensively summarized by Kümmerer (2004). However, some compounds, such as agents used in control of ticks and flies, are extensively used in CAFOs. A comprehensive list of US approved compounds for animals can be found in the Code of Federal Regulations (CFR 2003).

Table 1.2 Estimated yearly steroid hormone excretion by farm animals in the European Union and USA – year 2000 (Lange et al. 2002)

	European Union				USA			
Species	Million heads	Estrogens (tons)	Androgens (tons)	Gestagens (tons)	Million heads	Estrogens (tons)	Androgens (tons)	Gestagens (tons)
Cattle	82	26	4.6	185	98	45	1.9	253
Pigs	122	3.0	1.0	79	59	0.83	0.35	22
Sheep	112	1.3		58	7.7	0.092		3.9
Chickens	1002	2.8	1.6		1816	2.7	2.1	
Total	1318	33	7.1	322	1981	49	4.4	279

Table 1.3 Pharmaceuticals used extensively or uniquely in CAFOs

Compound	Classification	Use	CAS
Amitraz	Parasiticide	Mites and tick control	33089-61-1
Azamethiphos	Organic Phosphate	Flea and fly control	35575-96-3
Cyfluthrin	Pyrethroid insecticide	Fly control	68359-37-5
Cyhalothrin	Pyrethroid insecticide	Fly control	68085-85-8
Cypermethrin	Pyrethroid insecticide	Tick, mites and fly control	52315-07-8
Diazinon	Organic Phosphate	Flea and fly control	333-41-5
Flumethrin	Pyrethroid insecticide	Tick, mites and fly control	69770-45-2
Ivermectin	Parasiticide	Acaricide	70288-86-7
Lasalocid	Ionophore	Growth promoter in cattle; coccidiostat in poultry	25999-20-6
Malachite green	Dye	Anti-algal agent	569-64-2
Methomyl	Carbamate	Flea and fly control	16752-77-5
Methyltestosterone	Steroid	Sex reversal in fish	58-18-4
Monensin	Ionophore	Growth promoter in cattle; coccidiostat in poultry	22373-78-0
Narasin	Ionophore	Growth promoter in cattle; coccidiostat in poultry	55134-13-9
Norethisterone	Steroid	Sex reversal in fish	68-22-4
Roxarsone	Organic arsenic acid	Growth promoter in chickens	121-19-7
Permethrin	Pyrethroid insecticide	Fly control	52645-53-1
Pyriproxyfen	Insect growth factor	Flea and fly control	95737-68-1
Salinomycin	Ionophore	Growth promoter in cattle; coccidiostat in poultry	53003-10-4
Trenbolone	Steroid	Growth enhancer cattle	10161-33-8
Zearalenol	Resorcinol	Growth enhancer cattle	26538-44-3

1.4 Scope of the Book

The present treatise examines how hormones, antibiotics and pharmaceuticals generated from CAFOs are transported in water and soil. Little is known of the environmental fate of the tons of physiologically active steroid hormones released each year. In addition, in their own regard, in the last twenty years considerable attention has been given to a wide variety of natural and anthropomorphic agents known as endocrine disrupting compounds (EDCs). Until the overall contributions of steroid hormones to the environment are characterized, it will be difficult to quantify the additional effect of EDCs. Some information about the fate of these compounds in water has been obtained, but virtually nothing is known about how EDCs are transported in soil or by what mechanisms they eventually reach groundwater. As will be discussed extensively, it is somewhat of a mystery how the steroids; with their lipophilic nature, strong binding to humic acids and extensive metabolism by soil bacteria, can even be transported through more than a few centimeters of soil, let alone 20–40 m to the groundwater. The environmental fate of antibiotics through sewage treatment plants and water has been described previously (Kümmerer 2004). The emphasis in this treatise will focus more specifically on the emergence of antibiotic resistant bacteria in soil and the effects they may have on soil ecology, e.g., the presence of antibiotics preventing the metabolism of other active agents. Similarly the acaricides and insecticides used in animal husbandry are used widely for general use and their environmental pathways have been documented. The emphasis here again will be on the effects on soil and dung ecology. The compounds that are primarily generated from CAFOs will also be described, but little is known of their environmental fate so emphasis we be placed on defining the gaps in our knowledge and their possible impacts.

References

CFR (Code of Federal Regulations) (2003) Animal drugs, feeds, and related products. US Government Printing Office, Washington DC, 21 CFR 500, pp 500–599

EPA (2007) Regulatory definitions of large CAFOs, medium CAFO, and small CAFOs. http://www.epa.gov/npdes/pubs/sector_table.pdf [Web link verified Aug. 7, 2008]

Kümmerer K (ed) (2004) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn. Springer, Heidelberg Berlin

Lange IG, Daxenberger A, Schiffer B, Witters H, Ibarreta D, Meyer HHD (2002) Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. Anal Chim Acta 471:27–37

Oenema O (2004) Governmental policies and measures regulating nitrogen and phosphorus from animal manure in European agriculture. J Anim Sci 82:E196–E206

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C (2006) Livestock's long shadow. Environmental issues and options. Food and Agricultural Organization (FAO), Rome, 399 pp

Wood CL (1980) The Kansas beef industry. Lawrence: Regents Press of Kansas p. 286, ISBN 070060197X 978070060

Chapter 2
Steroid Hormones and Enzymes

Laurence Shore

Abstract This chapter provides a description of hormones, particularly androstenedione, progesterone, testosterone, estradiol and estrone, their physiochemical parameters, target organs and ubiquity in nature. The physiologically active steroids are produced from cholesterol. The two principal male hormones are testosterone and androstenedione and the two principal female hormones are estrone and estradiol. The main sources are cattle > poultry > swine manure. The hormones in the manure depend on the mode of excretion by the animal.

2.1 Steroidogenesis

Knowledge of the pathways of mammalian steroidogenesis was developed over the last 50 years and has resulted in a plethora of nomenclature. The description here is based on that of Norris (2007). The steroids are produced from cholesterol which may be ingested or synthesized in the body. The initial removal of the cholesterol side chain is by the side-chain cleaving enzyme, P450scc resulting in pregnenolone (P5). Δ5,3β-Hydroxysteroid dehydrogenase (3β-HSD) converts the P5 to progesterone (P4) (Fig. 2.1). The action of the 3β-HSD produces products through the Δ4 pathway. If pregnenolone is metabolized directly is referred to the Δ5 pathway. P4 and P5 are then converted either to DHEA (dihydroepiandrosterone) or androstenedione by C_{17} hydroxylase. Androstenedione (A) is converted to testosterone (T) by 17β-hydroxysteroid dehydrogenase (17β-HSD) (Fig. 2.1). The P450 aromatase then converts A or T to estradiol (E2) or estrone (E1) (Fig 2.2). 5α-Reductase can convert a double bond in steroid nucleus (*–ene*) group from most of the steroid pathways resulting in unsaturated *–ane* groups

L. Shore (✉)
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute, POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_2,

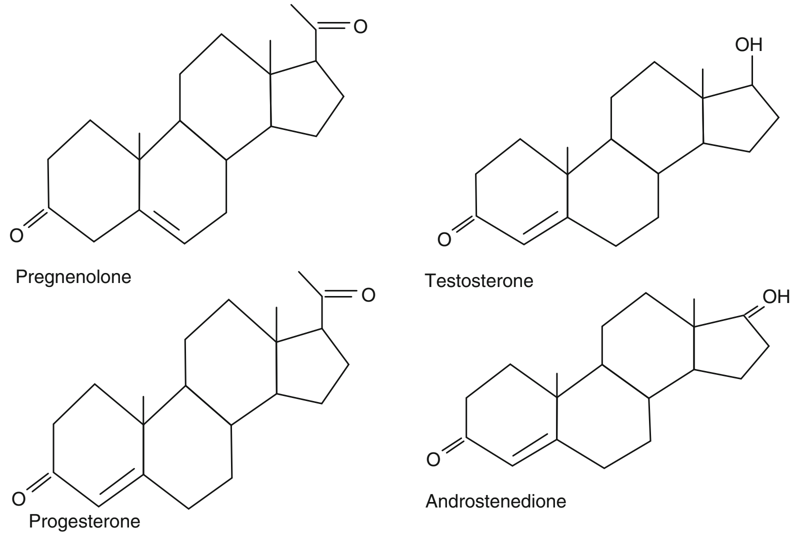


Fig. 2.1 Structure of steroids produced by domestic animals (progestins and androgens)

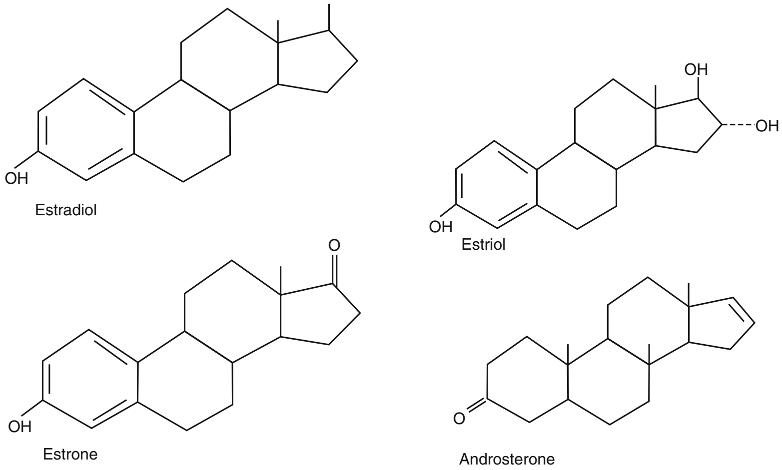


Fig. 2.2 Structure of steroids produced by domestic animals (estrogens and androsterone)

which generally results in an inactive molecule but some may be even more active (dihydrotestosterone from T).

Progesterone and its metabolites are termed progestins or gestatins since they maintain pregnancy in the mammal. Estrogens are defined as compounds that increase murine uterine weight. E2 and E1 are the principal estrogens in mammals. Compounds with progesterone or estrogen properties are also defined by their ability to bind their respective receptors. The principal androgens that are responsible for male characteristics, including development of reproductive organs, spermatogenesis and secondary sexual characteristics, are A and T. The androgens bind to the androgen receptor (AR). However the steroids, particularly E2, have non-genomic actions as well mediated through transmembrane receptors (Simoncini and Genazzani 2003).

The physiologically active steroids are constantly produced in the body by the gonads and adrenal glands. In the liver, they may be conjugated or metabolized to inactive compounds to make them more soluble so they can be excreted in the urine. However, the conjugated steroids are also excreted in the bile. Some of the steroids, in particular estrogens are deconjugated by intestinal bacteria and are reabsorbed. This process of recycling steroids is known as the entero-hepatic circulation (Borisenkov 2000; Renquist et al. 2008).

Mammals differ in how the steroids leave the body. In sheep, 77% of the P4, 44% of T and 89% of the E2 are excreted in the feces while in the pig the values are 34%, 14% and 4% (Palme et al. 1996). The cow is similar to the sheep (Estergreen et al. 1977; Mellin and Erb 1966).

2.2 Steroids Produced by Cows

The major progesterone metabolites excreted by cows are 3α-hydroxy-5β-pregnan-20-one; 3β-hydroxy-5β-pregnan-20-one; 5β-pregnane-3α,20β-diol and 5a-pregnane-3β, 20β-diol (Miller 1996). In sheep the major progestins are 3α-hydroxy-5β-pregnan-20-one; 3β-hydroxy-5β-pregnan-20-one; 5α-pregnane-3,20-dione and 3β-hydroxy-5α-pregnan-20-one (Miller 1996). Comparatively, very little of the progesterone is secreted as the active agent, progesterone (Isobe et al. 2005). Estrogens are excreted as 17α-estradiol, E2, E1 and estriol (E3) (Fig. 2.2). In cows the principal androgens are 17α-testosterone, T and A.

2.3 Steroids Produced by Pigs

Pigs produces the same steroids as cows. Quantitatively, the major estrogens are E1 and E3 (Furuichi et al. 2006). Boars excretes a special steroid in its saliva called androstenone (5α-androst-16-en-4-one) (Fig. 2.2).

2.4 Steroids Produced by Poultry

Chickens, turkeys and ducks excrete E1, E2, T and A (Shore et al. 1988, 1993). There is little difference in T and E between male and female broilers (chickens raised for meat until 8–10 weeks of age) (see Chap. 3).

2.5 Steroids Excreted by Fish

Fish plasma steroid profiles are similar to mammals with the major difference in that the principal androgen is 11-ketotestosterone. Of particular importance to environmental effects are the pheromones produced by fish since they can act in ng/l concentrations. Androstenedione, 17,20-dihydroxypregn-4-en-3-one and 17,20-dihydroxypregn-4-en-3-one-20-sulfate are pheromones in goldfish (*Carassius auratus*) (Scott and Sorensen 1994); T in salmon (*Salmo salar*) (Moore and Scott 1991); E2 and E1 in the round goby (*Neogobius melanostomus*) (Murphy et al. 2001), 3α-hydroxyl, 5β-androstan-17-one-3α-glucoronide in the black goby (*Gobius jozo*) (Colombo et al. 1980), 3α,17α-dihydroxy-5β-pregnan-20-one-3α-glucoronide in the catfish (*Clarias gariepinus*) (Resink et al. 1989) and 17α,20β-dihydroxy-4-pregnen-3-one in *Barilius bendelisis* (Bhatt and Sajwan 2001).

2.6 Steroids Present in Terrestrial Invertebrates

In general it is accepted that many of the same pathways for steroidogenesis exist in invertebrates as do in the vertebrates (Flari and Edwards 2003). For example, evidence of endogenous changes in E2, P4 and T correlated with the reproductive cycle has been reported for two hermaphroditic desert snails (Alon et al. 2007). Due the widespread distribution of tributyltin and its wide ranging effects on reproductive organs of marine mollusks, there have been several studies showing that many mollusks, crustaceans and echinoderms have 3β-HSD, 17β-HSD, aromatase and 5α reductase (Janer et al. 2005). In general, neither functional steroid receptors nor non-genomic transmembrane receptors have been demonstrated in invertebrates (Keay et al. 2006; Zhu et al. 2003).

Insects, as do some worms and crabs, use a steroid, 20-hydroxyecdysone, to regulate many functions but this steroid does not have common biochemical pathways with the mammalian reproductive steroids. Receptors to 20-hydroxyecdysone are inhibited by a group of compounds called Insect Growth Regulators (IGRs) which are widely used by CAFOs (see Sect. 13.1.3). However, the silkworm (*Bombyx mori*) does have endogenous estrogen and estrogen binding proteins (Keshan and Ray 2001).

2.7 Steroidogenic Enzymes in Bacteria and Fungi

17β-HSDs reversibly catalyze the conversion of A to T and E2 to E1. It is present in many bacteria and several fungi (Baker 1991; Rizner and Zakelj-Mavric 2000). The presence of 17β-HSDs are thought to be responsible for the rapid change from E2 to E1 which takes place in the fecal matter and soil (Chaps. 3 and 6).

Other enzymes found in some bacteria are 3α,20β-HSD and 3β,17β-HSD. The function of these enzymes is thought to be to protect bacteria and fungi from the toxic effects of steroids or sterols (Breskvar et al. 1995; Jeraj et al. 2003).

References

Alon G, Shore LS, Steinberger Y (2007) Correlation between levels of sex hormones (progesterone, testosterone, estrogen) and ecophysiological behavior stages in two species of desert snails (*Sphincterochila zonata* and *Sphincterochila prophetarum*) in the northern Negev Desert. Gen Comp Endocrinol 151:122–127

Baker ME (1991) Genealogy of regulation of human sex and adrenal function, prostaglandin action, snapdragon and petunia flower colors, antibiotics and nitrogen fixation; functional diversity from two ancestral dehydrogenases. Steroids 191:354–360

Bhatt JP, Sajwan MS (2001) Ovarian steroid sulphate functions as priming pheromone in male *Barilius bendelisis* (Ham.). J Biosci 26:253–263

Borisenkov MF (2000) The content of sex hormones in the enteric medium of female ruminants. J Evol Biochem Phys 36:59–65

Breskvar K, Ferencak Z, Hudnik-Plevnik T (1995) The role of cytochrome $p450_{11}^{\alpha}$ in detoxification of steroids in the filamentous fungus *Rhizopus nigricans*. J Steroid Biochem Mol Biol 52:271–275

Colombo L, Marconato A, Belvedere PC, Friso C (1980) Endocrinology of teleost reproduction: a testicular steroid pheromone in the black goby *Gobius jozo L.* Boll Zool 47:355–364

Estergreen VL, Lin MT, Martin EL, Moss GE, Branen AL, Luedecke LO, Shimoda W. (1977) Distribution of progesterone and its metabolites in cattle tissues following administration of progesterone-4-C^{14}. J Anim Sci 45:642–651

Flari VA, Edwards JP (2003) The role of the endocrine system in the regulation of reproduction in terrestrial pulmonate gastropods. Invertebr Reprod Dev 44:139–161

Furuichi T, Kannan K, Suzuki K, Tanaka S, Giesy JP, Masunaga S (2006) Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant. Environ Sci Technol 40:7896–7902

Isobe N, Akita M, Nakao T, Yamashiro H, Kubota H (2005) Pregnancy diagnosis based on the fecal progesterone concentration in beef and dairy heifers and beef cows. Anim Reprod Sci 90:211–218

Janer G, LeBlanc GA, Porte C (2005) A comparative study on androgen metabolism in three invertebrate species. Gen Comp Endocrinol 143:211–221

Jeraj N, Romih R, Lenasi H, Breskvara K (2003) In situ detection of progesterone binding sites in the plasma membrane of the filamentous fungus *Rhizopus nigricans*. Acta Chim Slov 50:757–762

Keay J, Bridgham JT, Thornton JW (2006) The *Octopus vulgaris* estrogen receptor is a constitutive transcriptional activator: evolutionary and functional implications. Endocrinology 147: 3861–3869

Keshan B, Ray AK (2001) The presence of estradiol-17β and its specific binding sites in posterior silk gland of *Bombyx mori*. Gen Comp Endocrinol 123:23–30

Mellin TN, Erb RE (1966) Estrogen metabolism and excretion during bovine estrous cycle. Steroids 7:589–606

Miller CW (1996) Progestin analysis by gas chromatography/mass spectrometry in plasma and feces of horses, cattle, and sheep. Thesis, Oregon State University, Corvallis, OR

Moore A, Scott AP (1991) Testosterone is a potent odorant in precocious male Atlantic salmon (*Salmo salar* L.) Parr. Philos Trans R Soc B 332:241–244

Murphy CA, Stacey NE, Corkum LD (2001) Putative steroidal pheromones in the round goby, *Neogobius melanostomus*: olfactory and behavioral responses. J Chem Ecol 27:443–470

Norris DO (2007) Vertebrate endocrinology, 4th edn. Elsevier Academic Press, Burlington, MA
Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 43:43–63
Renquist BJ, Adams TE, Adams BM, Calvert CC (2008) Dietary restriction reduces the rate of estradiol clearance in sheep (*Ovis Aries*). J Anim Sci 34:301–310
Resink JW, Schoonen WGEJ, Albers PCH, File DM, Notenboom CD, Van Den Hurk R, Van Oordt PGWJ (1989) The chemical nature of sex attracting pheromones from the seminal vesicle of the African catfish, *Clarias gariepinus*. Aquaculture 83:137–151
Rizner TL, Zakelj-Mavric M (2000) Characterization of fungal 17β-hydroxysteroid dehydrogenases. Comp Biochem Physiol B Biochem Mol Biol 127:53–63
Scott AP, Sorensen PW (1994) Time course release of phernomally active gonadal steroids and their conjugates by ovulatory goldfish. Gen Comp Endocrinol 96:309–323
Shore LS, Shemesh M, Cohen R (1988) The role of oestradiol and oestrone in chicken manure silage in hyperoestrogenism in cattle. Aust Vet J 65:67
Shore LS, Harel-Markowitz E, Gurevich M, Shemesh M (1993) Factors affecting the concentration of testosterone in poultry litter. J Environ Sci Health A 28:1737–1749
Simoncini T, Genazzani AR (2003) Non-genomic actions of sex steroid hormones. Eur J Endocrinol 148:281–292
Zhu Y, Bond J, Thomas P (2003) Identification, classification, and partial classification of genes in humans and other vertebrates homologous to fish membrane progestin receptor. Proc Natl Acad Sci USA 100:2237–2242

Chapter 3
Steroid Hormones Generated by CAFOs

Laurence Shore

Abstract The steroid hormones are phylogenetically very old and nature has developed numerous mechanisms for their decomposition. However, the huge volume of steroids generated from CAFOs and the appearance of hormones in streams and rivers requires that possible environmental impact needs to be assessed.

3.1 Sampling

There is significant concern about spreading disease between herds, especially swine and poultry, and steps should be taken when making observations at animal feeding operations. Researchers that visit one farm should not be allowed to visit another farm without letting 2 days elapse. Additionally, industry hygiene standards should be adopted when visiting each farm, such as placing disposable plastic covers over shoes and wearing disposable gloves.

Precautions should also be made in taking environmental samples so that hormone attenuation is not facilitated. Fine et al. (2003) found that pre-treatment of liquid manure samples with formaldehyde was necessary to prevent conversion of E2 to E1, and that acidification of samples to pH 2 (Raman et al. 2004) can cause E1 to precipitate. However slight acidification to pH 4–5 is acceptable as is the addition of 0.1% sodium azide (Shore, personal observation). Formaldehyde pre-treatment is not needed for the ground water samples (Fine et al. 2003). Sample storage devices and instruments need all to be constructed of materials that do not sorb hormones or other compounds likely to be present in the manure. Batch experiments conducted with ^{14}C labeled E2, E1, and T indicated that stainless steel, glass, and polypropylene are suitable materials (Thompson 2005). Also, samples need to be placed

L. Shore (✉)
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute, POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_3,

immediately in a cooler with ice and covered to prevent photodegradation. Sample analysis should be done in a timely manner.

3.2 Measurement

Analytical methods that can determine part-per-trillion level hormone concentrations from complex matrices, such as soil and manure, are required. Preliminary sample screening for the presence of hormones can be done using a modified yeast estrogen screening assay (YES) (Lorenzen et al. 2004; Soto et al. 1995). Methods are available to extract and purify samples so that analyses for androgenic and estrogenic hormones using liquid chromatography (LC) with tandem mass spectrometry (MS-MS) may be performed (Casey et al. 2005; Fine et al. 2003; Kolodziej et al. 2003; Raman et al. 2004). The LC-MS-MS method used is preferred and an example method is described in Casey et al. (2005). In brief, the first MS of the tandem MS system is used to identify the molecular ion, and the second MS identifies and quantifies fragment ions. This use of the MS-MS leads to high confidence in molecular identification and quantification. However, all of these methods can still have matrix effects when measuring complex matrices such as slurry and lagoons (Díaz-Cruz et al. 2003; Fine et al. 2003; Díaz-Cruz and Barceló 2007).

Because steroid analysis is so important to human medicine, there are many commercial immunoassay kits available. Immunoassays are very sensitive and simple to use but specificity of the assay is limited by the antibody purity, i.e., antibodies cross-react to some extent with other steroids. Although the cross reactions are usually in the order of less than 1%, the large amount of steroids present in manures and slurries can still have an effect. For example, progesterone (P4) is not present in detectable levels in cycling dairy cow manure as measured in GC/MS (Miller 1996) but the P4 antibody cross-reacts enough with the pregnandiones and pregnandiols (Chap. 2), to give a P4 value of about 200 ng/g (which can be still be used to characterize the cow's cycle).

3.3 Extraction

Although manures are by nature a heterogeneous matrix, extraction is simpler than from tissue or sera as there are no binding proteins. Simple organic solvents such as methanol and ethyl acetate or even water can be used. This is followed by cleanup on solid phase extraction (SPE) columns, usually C-18. After passing the sample through the column, the column is then eluted with methanol. For many kits the methanol extract in a 40% solution can then be assayed directly. Slurry, that is the effluent from the barn or sty, presents other difficulties. The high lipid content of the dairy barn effluent makes extraction of the solid phase (about 30%) extremely difficult. One therefore needs to extract with non-polar solvents such as petroleum ether or dichloromethane and evaporate the extract to dryness. The residue is then taken up in methanol for SPE columns and the column washed with solvents such as hexane before elution. Although most of the estrone produced by gravid ruminants is the form of estrone sulfate, in ruminants and swine feces, essentially all of the steroid hormones, including E1 are unconjugated (Schwarzenberger et al. 1996). Therefore, treatment with sulfatase/glucoronidase is generally not necessary to measure total estrogen.

3.4 Steroid Production by Cattle, Swine and Poultry

3.4.1 Production by Cattle

Due to the widespread use of manure management programs, the amount of manure produced by various CAFOs is well documented so it is possible to estimate the total amount of manure produced and the total steroid produced if the steroid concentration is known (The Ohio State University Extension 2006; Table 1.2). For example, in ruminants 90% of the estrogen is produced in the feces and only about 10% in the urine (Palme et al. 1996). Cows produces 70 L/day of effluent (7 kg solids) and 30 L of this is urine.

Although bulls produce estrogen (9 μg/kg dry manure) and other steroids, dairy farms use artificial insemination (AI) so bulls would not significantly contribute to the hormone released. Furthermore, essentially no estrogen (<2 μg/kg dry wt) was found in slurry from bulls (Wenzel et al. 1998). The ratio of bulls to cows on pasture is usually 1:20 but bulls do not excrete more androgens than cows (Turner 1947).

Most of the estrogen produced by the cow is during the last 3 months of pregnancy (Table 3.1). In CAFOs about 60% of the cows are pregnant during the year. T and A are present in dairy manure piles in about the same concentration (25 μg/kg). 17α-Estradiol is quantitatively the major estrogen produced in ruminants and accounts for 80% of the estrogen (Hoffmann et al. 1999). 17α-Estradiol is considered a very weak estrogen and therefore is not of environmental concern, although it does have physiological effects in some systems (Hajek et al. 1997). The progesterone metabolites, pregnanolones and pregnandiols, are produced in relatively large quantities (40 μg/g for the pregnant cow and 120 μg/g for the pregnant ewe; ewes are higher due to multiple gravidities) (Miller 1996), but these 5α reduced compounds do not have progesterone action. However, as discussed in Chap. 2, these compounds are pheromones in some fish so there is some potential for environmental disturbance. Although in general, progesterone in the absence of estrogen has no physiological effects, there may be an indirect environmental effect as progesterone produced by pine trees is converted in sufficient quantities in wood pulp processing plants to androstenedione to have effects on fish (Carson et al. 2008).

Table 3.1 Estrogen concentrations in dairy cow effluent

Source	Estrone (μg/kg)	Estradiol 17β (μg/kg)	Comments	Ref.
Milk cows (slurry)	255–640	170–1,230	Total solids	Wenzel et al. (1998)
Bulls (slurry)	<2	<2	Total solids	Wenzel et al. (1998)
Milk cows (feces, late gestation)	840 (estrone + estradiol)		Dry wt	Möstl et al. (1997)
Milk cows (manure pile)	700–1,000 (estrone + estradiol)		Dry wt	Möstl et al. (1997)
Milk cows (manure pile)	28–72	120–190	Dry wt	Vethaak et al. (2002)
Milk cows (feces)				
100 days before parturition	0.9	9.0	Fresh wt	Hoffmann et al. (1999)
60 days before parturition	0.1	13.9	Fresh wt	Hoffmann et al. (1999)
30 days before parturition	4.1	19.1	Fresh wt	Hoffmann et al. (1999)
10 days before parturition	9.4	42.2	Fresh wt	Hoffmann et al. (1999)
5 days before parturition	11.4	60.0	Fresh wt	Hoffmann et al. (1999)

3.4.2 Production by Swine

Most recent quarterly statistics in the US indicate that there are over 62 million swine currently in production, and that production has steadily increased since 2000 at a rate of ~1 million swine per year (USDA-NASS 2006). Hormone production varies widely among species, but also between individuals within a species. Although in 2006 US swine meat production (~9.5×10^9 kg [2.1×10^{10} lbs]) was lower than beef (~1.2×10^{10} kg [2.6×10^{10} lbs]) and poultry (~1.6×10^{10} kg [3.6×10^{10} lbs]) (USDA-ERS 2007), swine may contribute significant concentrations of hormones into the environment. Within swine, hormone production varies by sex (e.g., boar, sow) and reproductive stage (e.g., pregnant), and operations segregate animals into gestation, farrowing (where pigs are born and weaned), nursery (where young pigs are kept for ~40–70 days), and finishing facilities (where pigs are kept until they reach a specific market weight). The amount and type of hormones found in the manure holding systems at these facilities will depend on the facility type (e.g. there would be little T in barns holding castrated males). Manure from swine gestation and farrowing facilities, where pregnant sows produce large amounts of E2, has a significant potential to produce and contribute estrogenic compounds to the environment (Fine et al. 2003; Lorenzen et al. 2004; Raman et al. 2004). In Europe, due to pressure to reduce the number of animals in CAFOs, AI is used in more than 90% of the CAFOs, while in the US, AI is used in less than 40% of the farms.

Manure at a swine farm is handled either as a solid or a liquid. Most manure is handled as a liquid and more liquid manure is produced in gestation, farrowing, and nursery facilities. Usually, liquid manure is held in Manure Storage Ponds (MSP) or lagoons. Lagoons encourage anaerobic digestion of organics while MSPs do not. Liquid manure is agitated to make the nutrient content uniform before it is applied to the land by surface application, injection, or irrigation. The majority of solid manure is produced in finishing barns and is usually a mixture of bedding and manure. The bedding and manure is periodically scraped from finishing barns (~every 6 months) and then piled uncovered (sometimes covered) on a pad or land surface. This manure pile is then spread on the land in spring or fall when the crop is off the field.

Hormones in manure have been quantified for nonpregnant and pregnant sows and during different gestational stages, and for different manure holding systems (Table 3.2). In addition to the rise in estrogen towards parturition seen in ruminants, the sow has a peak of estrogen level about 28 days after inception (Vos 1996). Among the estrogens (i.e., E2 and its two metabolites, E1 and estriol), E1 is found in highest concentrations in manures (finishing lagoons max E1 = 74.7 μg/L, farrowing lagoons max E1 = 25.7 μg/L, nursery lagoons max E1 = 0.58 μg/L) (Fine et al. 2003). Concentrations of E2 and E1 have also been found to increase with depth in MSPs (Raman et al. 2004) which may be attributed to partitioning to organic solids that settled to the bottom, or to a decrease in degradation potential as anaerobic conditions increase with depth. Raman et al. (2004) calculated ratios of estrogen to macronutrient content (N, P, and K) in various manures, which is important because application rates are determined by soil nutrient requirements.

Table 3.2 Estrogen concentrations in swine effluent

Source	Estrone (μg/kg or μg/L)	Estradiol 17β (μg/kg or μg/L)	Comments	Ref.
Swine (slurry)	<2–84	<2–64	Dry wt	Wenzel et al. (1998)
Sow urine (early gestation)	65			Choi et al. (1987)
Sow feces (early gestation)	35		Fresh wt	Choi et al. (1987)
Sow feces (early gestation)	9	8	Fresh wt	Vos (1996)
Sow feces (early gestation)	10 (E1 sulfate)	2.3 (E2 sulfate)	Fresh wt	Vos (1996)
Sow (feces, late gestation)	15–28		Dry wt	Hoffman (personal comm.)
Raw wastewater	5.2	1.0		Furuichi et al. (2006)
Finishing lagoon	10	2.5		Raman et al. (2004)
Farrowing pit	55	37		Raman et al. (2004)
Farrowing pit	4,900	1,500	Total solids	Raman et al. (2004)
Farrowing lagoon	55	3.9		Raman et al. (2004)
Farrowing lagoon	600	900	Total solids	Raman et al. (2004)
Farrowing lagoon	17	2.5		Furuichi et al. (2006)

Table 3.3 Hormone concentration in chicken manure

Source	T (μg/kg d.w.)	E (μg/kg d.w.)	Ref.
Immature broilers			
Females	133	65	Shore and Shemesh (1993)
Males	133	14	Shore and Shemesh (1993)
Laying hens	254	533	Shore and Shemesh (1993)
Roosters	670	93	Shore and Shemesh (1993)
Chicken litter		133	Nichols et al. (1997)

Estrogen:macronutrient ratios from swine farrowing manure were found to be 4× greater than dairy manure, even though the later included manure from lactating cows in various stages of their reproductive cycle. Swine and other monogastric mammals excrete most of their hormones in the urine as opposed to the ruminants which excrete most of the hormones in the feces (Palme et al. 1996).

3.4.3 Production by Poultry

Chicken CAFOS include egg layers, breeders and broiler operations. In broiler CAFOs, chicks are raised to 8–10 weeks of age before marketing. With broilers, the entire coop is cleaned out after the chickens are marketed. Similarly, egg laying operations are usually cleaned out after the hens have completed one or two laying cycles. Like most birds, there is little difference in the testosterone and estrogen (Table 3.3) content between males and females particularly before age of egg laying at 5–6 months. Breeder operations maintain a rooster:hen ratio of about 1:10. Chicken manure mixed with the floor scrapings of the coop is referred to as chicken litter. Turkey and duck manure has about only about 10% of hormone levels found in chicken litter.

3.5 Steroid Stability in Manures

In liquid form, the steroid hormones in manure are rapidly metabolized. For example, the half time of fecal steroids excreted into a duck pond is less than an hour and after 20 years, there was no evidence of accumulation in the pond sediment. However, if the manure is dry (<40%), the available steroid concentration tends to increase due to microbial activity (Millspaugh and Washburn 2004; Shore and Shemesh 1993; Shore et al. 1993; Table 3.4). Silaging the manure anaerobically with wheat or corn has little effect on the steroid levels (Table 3.5) but composting (aerobic degradation) reduces the level to below 10 ng/g T or E2 (80–90% reduction) (Hakk et al. 2005).

Table 3.4 Androstenedione (A) and testosterone (T) in cattle manure, 1 and 3 months after formation of pile. Ten sites were sampled at each occasion

	μg/kg (after 1 month)	μg/kg (after 3 months)	1 month vs. 3 months
T	15.9 ± 2.4	25.2 ± 6.9	P < 0.01
A	13.9 ± 2.1	17.8 ± 2.6	P < 0.01
T vs. A	P < 0.03	P < 0.01	

Table 3.5 Effect of silaging on chicken manure. Poultry litter was tested before and after silaging. Each mean ± SD represents sampling from five farms. The manure was sampled in duplicate, each duplicate consisting of a pool of ten sites from the pile

Hormone in μg/kg	Before silaging	After silaging	
Androstenedione	149 ± 69	101 ± 39	NS
Estrogen	38 ± 11	55 ± 14	P < 0.03
Testosterone	68 ± 17	102 ± 17	P < 0.01

Table 3.6 Hormone concentrations of testosterone (T), estrogen (estradiol + estrone; E1 + E2), androstenedione (A) and estriol in an aquaculture pond. Hormone values were measured at three depths in the months following the introduction of hatchlings. The pond had about 100,000 male and female carp, but only the females developed gonads in the first year

	T (ng/L)	E1 + E2 (ng/L)	A (ng/L)	Estriol (ng/L)
One month after	2.37	0.97	1.62	<0.1
Five months after	3.75	2.20	4.67	<0.1
Eight months after	5.73	7.07	18.23	<0.1

3.6 Steroids Produced in Aquaculture

Aquaculture ponds accumulate large quantities of steroids and these are of concern as the ponds are usually emptied at the end of the season and the effluent is directly released into surface water or used for irrigation (Kolodziej et al. 2004; Vethaak et al. 2002). Unlike animal CAFOs there is little information on the amounts involved since aquaculture is not included in manure management programs. The principle androgen produced by carp species is androstenedione (A) as this compound is a pheromone in carp species (Sorensen et al. 2005) (Table 3.6).

References

Casey FXM, Šimůnek J, Lee J, Hakk H, Larsen GL (2005) Sorption, mobility, and transformation of estrogenic hormones in natural soil. J Environ Qual 34:1372–1379

Carson JD, Jenkins RL, Wilson EM, Howell WM, Moore R (2008) Naturally occurring progesterone in loblolly pine (*Pinus taeda* l.): a major steroid precursor of environmental androgens. Environ Toxicol Chem 27:1273–1278

Choi HS, Kiesenhofer E, Gantner H, Hois J, Bamberg E (1987) Pregnancy diagnosis in sows by estimation of oestrogens in blood, urine or faeces. Anim Reprod Sci 15:209–216

Díaz-Cruz MS, Barceló D (2007) Recent advances in LC-MS residue analysis of veterinary medicines in the terrestrial environment. Trends Anal Chem 26:637–646

Díaz-Cruz MS, López de Alda MJ, Barceló D (2003) Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. Trends Anal Chem 22:340–351

Fine DD, Breidenbach GP, Price TL, Hutchins SR (2003) Quantitation of estrogens in ground water and swine lagoon samples using solid-phase extraction, pentafluorobenzyl/trimethylsilyl derivatizations and gas chromatography–negative ion chemical ionization tandem mass spectrometry. J Chromatogr A 1017:167–185

Furuichi T, Kannan K, Suzuki K, Tanaka S, Giesy JP, Masunaga S (2006) Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant. Environ Sci Technol 40:7896–7902

Hakk H, Millner P, Larsen G (2005) Decrease in water-soluble 17β-estradiol and testosterone in composted poultry manure with time. J Environ Qual 34:943–950

Hajek RA, Rober AD, Johnston DA, Van NT, Tcholakian RK, Wagner LA, Conti CJ, Meistrich ML, Contreras N, Edwards CL, Jones LA (1997) During development, 17 α-estradiol is a potent estrogen and carcinogen. Environ Health Prospect 105(Suppl. 3):577–581

Hoffmann B, Gopes de Pinho T, Shuler G (1999) Determination of free and conjugated oestrogens in peripheral blood plasma, feces and urine of cattle throughout pregnancy. Exp Clin Endocrinol Diabetes 105:296–303

Kolodziej EP, Gray JL, Sedlak DL (2003) Quantification of steroid hormones with pheromonal properties in municipal wastewater effluent. Environ Toxicol Chem 22:2622–2629

Kolodziej EP, Harter T, Sedlak DL (2004) Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ Sci Technol 38:6377–6384

Lorenzen A, Hendel JG, Conn KL, Bittman S, Kwabiah AB, Lazarovitz G, Massé D, McAllister TA, Topp E (2004) Survey of hormone activities in municipal biosolids and animal manures. Environ Toxicol 19:216–225

Miller CW (1996) Progestin analysis by gas chromatography/mass spectrometry in plasma and feces of horses, cattle, and sheep. Thesis, Oregon State University, Corvallis, OR

Millspaugh JJ, Washburn BE (2004) Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen Comp Endocrinol 138:189–199

Möstl E, Dorbretsberger A, Palme R (1997) Östrogenkonzentration im stallmist trächtiger rinder. Wien Tierärztl Mschr 84:140–143

Nichols DJ, Daniel TC, Moore A, Edwards DR, Pote DH (1997) Runoff of estrogenic hormone 17β-estradiol from poultry litter applied to pasture. J Environ Qual 26:1002–1006

Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 43:43–63

Raman DR, Williams EL, Layton AC, Burns RT, Easter JP, Daugherty AJ, Mullen D, Sayler GS (2004) Estrogen content of dairy and swine wastes. Environ Sci Technol 38:3567–3573

Schwarzenberger F, Möstl E, Palme R, Bamberg E (1996) Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim Reprod Sci 42: 515–526

Shore LS, Harel-Markowitz E, Gurevich M, Shemesh M (1993) Factors affecting the concentration of testosterone in poultry litter. J Environ Sci Health A28:1737–1749

Shore LS, Shemesh M (1993) Analysis of testosterone levels in chicken manure. Isr J Vet Med 48:35–37

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO (1995) The E-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103:133–122

Sorensen PW, Pinillos M, Scott AP (2005) Sexually mature male goldfish release large quantities of androstenedione into the water where it functions as a pheromone. Gen Comp Endocrinol 140:164–75

The Ohio State University Extension (2006) Ohio livestock manure management guide 604. http://ohioline.osu.edu/b604/index.html. Accessed 7 Aug 2008

Thompson ML (2005) Persistence and pathways of 17β-estradiol in agricultural soils: a field study. Master's thesis, North Dakota State University, Fargo ND

Turner CW (1947) The male hormone content of ruminant manure. J Dairy Sci 30:1–8

USDA-ERS (2007) Livestock, dairy, and poultry outlook/LDP-M-152/Feb. 20 2007. Economic Research Service, Washington, DC. http://www.ers.usda.gov/Publications/LDP/2007/02Feb/ldpm152tables.pdf. Accessed 7 Aug 2008

USDA-NASS (2006) Quarterly hogs and pigs. Agricultural Statistics Board, Washington, DC. http://usda.mannlib.cornell.edu/usda/nass/HogsPigs//2000s/2006/HogsPigs-12-27-2006.pdf?bcsi_scan_9836791FF96CD3A5=0&bcsi_scan_filename=HogsPigs-12-27-2006.pdf. Accessed 7 Aug 2008

Vethaak AD, Rijs GBJ, Schrap SM, Ruiter H, Gerritsen A, Lahr J (2002) Estrogens and xeno-estrogens in the aquatic environment of the Netherlands RIZA/RIKZ-Report no. 2001.028. National Institute for Coastal and Marine Management (RIKZ), The Hague, Netherlands

Vos EA (1996) Direct ELISA for estrone measurement in the feces of sows: prospects for rapid, sow-side pregnancy diagnosis. Theriogenology 46:211–231

Wenzel A, Kuechler Th, Mueller J (1998) Konzentrationen oestrogen wirksamer Substanzen in Umweltmedien. Report. Project sponsored by the German Environmental Protection Agency, Project No. 216 02 011/11

Chapter 4
Transport of Steroids in Surface Waters

Laurence Shore

Abstract Testosterone, androstenedione and estrogens are found in run-off from cattle pasture and fields fertilized with manure. The distribution in the primary, secondary and tertiary branches of watersheds is described. In rivers, hormones from CAFOs can be traced over 60 km away.

4.1 Source of Steroids in Surface Waters

Most steroidal hormones from CAFOs reach streams and rivers in the run-off either from the CAFOs or manured fields. In the case of aquaculture, the effluent may be discharged directly into the surface water. The natural contribution of the fish population of lakes is measurable but less than 0.5 ng/l of testosterone or estradiol. It is difficult to find surface waters that are also not contaminated with sewage water treatment plant (SWTP) effluent, but the contribution of this effluent can be characterized by the ubiquitous presence of ethinylestradiol (EE) which is not used in CAFOs.

4.2 Run-Off from Cattle Pasture

Manure from cattle on grazing land can accumulate during the dry season typical of areas like Israel and California. When the winter arrives the initial rainfalls can result in steroid run-off into nearby streams. Shore et al. (2004) reported that this initial run-off contains estrogens (E1 + E2), T and androstenedione (A) in ng/l

L. Shore (✉)
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute, POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_4,

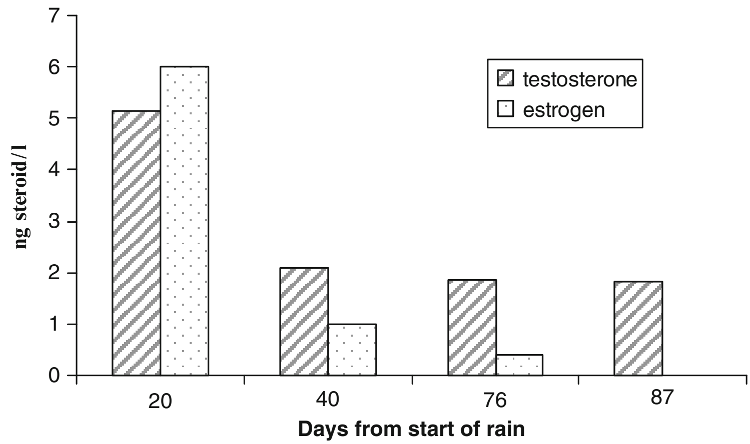


Fig. 4.1 Testosterone and estrogen in streams receiving run-off from cattle pasture at the start of the rainy season

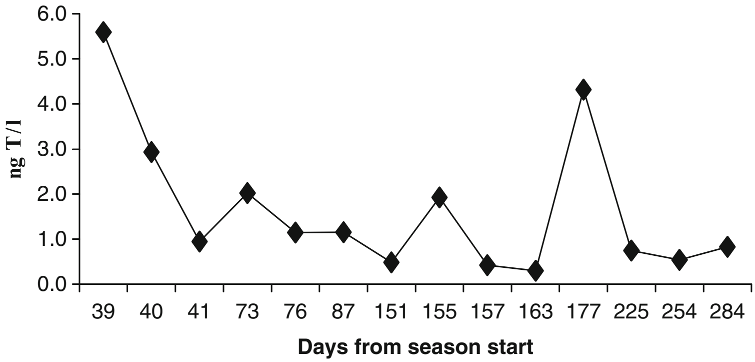


Fig. 4.2 Testosterone (T) pulses after each rain event in a river draining a watershed with cow pasture from 39 to 225 days after the start of the rainy season. In the dry season (days 254 and 284), T was less than 1 ng/l

quantities (Fig. 4.1). However 1 month after the start of the rainy season, only T was present in the streams. The evidence suggests that only T penetrates the soil and the T continues to wash out of the soil. In areas where there is a high water table, this effect is seen in receiving surface waters after each rain pulse (Fig. 4.2). In 57 sub-catchment areas, selected to reflect primarily the contribution of cattle on rangeland, testosterone above 1 ng/l (1.3–7.5 ng/l) was detected in over 90% of the samples while androstenedione (1.2–2.9 ng/l) was present in only 29%. Similarly, Jenkins et al. (2006) reported that a watershed with a density of about one cow-calf pair/ha 3 weeks after introduction, produced 39 ng T and 39 ng E/l in March and 147 ng/l E and 6.0 ng/l T in July after major rain events. This was in disagreement with the observations of Kolodziej and Sedlak (2007) who found that androstenedione above 1 ng/l (range 2.8–41 ng/l) was present in 6/89 samples in streams draining a California rangeland while testosterone was present in only one sample (2.3 ng/l).

4.3 Run-Off from Fields Manured with Chicken Manure or Litter

Following rain events both estrogen and testosterone are found in the run-off from fields manured with chicken manure. Edge of field run-off following rain events contained substantial amounts of estrogen and testosterone (1–3 μg/l) (Finlay-Moore et al. 2000; Nichols et al. 1997, 1998). The amount of estradiol in the run-off increased linearly with the increasing application rate of the litter (1.76–7.05 Mg litter/ha), increase in pH, and TOC (total organic carbon). The concentration and mass losses of E2 in the run-off can be remarkably reduced using buffer strips or addition of alum to the litter. In a pond on the Eastern Shore MD, which received run-off from a manured field, estrogen (E1 + E2) levels were above 5 ng/l (maximum 25 ng/l) for several months with a half-life of about 2.5 months (Shore et al. 1995). The run-off in receiving streams of the field normally had <1 ng T/l but immediately after the field was manured contained 21–34 ng T/l. Similarly, Jenkins et al. (2006) found that run-off from freshly poultry manured fields was 38.7–196 ng/l E2 and 3.3–7.4 ng/l T following rain events in watersheds in GA. Substantial quantities of estrogen can also elute from stack manure piles. It was found that elutants from chicken manure piles were found to contain 630 ng/l of testosterone and 730 ng/l of estrogen. In the Connestoga River watershed in central Pennsylvania, it was found that four streams (4/17) had 2.2–4.2 ng/l T and 0.8–2.9 ng/l E. Three of these sites were near fields amendated with chicken manure and one below an SWTP (Shore et al. 1995).

4.4 Transport in Rivers

Information on transport of steroids in rivers comes primarily from studies of effluent from SWTPs (Barel-Cohen et al. 2006; Jürgens et al. 1999, 2002; Labadie and Budzinski 2005; Shore et al. 2004; Williams et al. 2003). Unconjugated estrone is the main estrogenic compound detectable in rivers and it is removed primarily by biodegradation and the degradation is more complete at higher temperatures (Fig. 4.3). Absorption to sediment, suspended particulate matter, or photodegradation are not a major routes of estrogen removal. Dilution alone (as measured using boron) cannot explain the reduction of steroids. Estradiol and estriol disappear rapidly but testosterone can persist similar to estrone. Similarly as seen in SWTPs, biodegradation does not seem to reduce estrone or testosterone to below 1.5–2 ng/l,

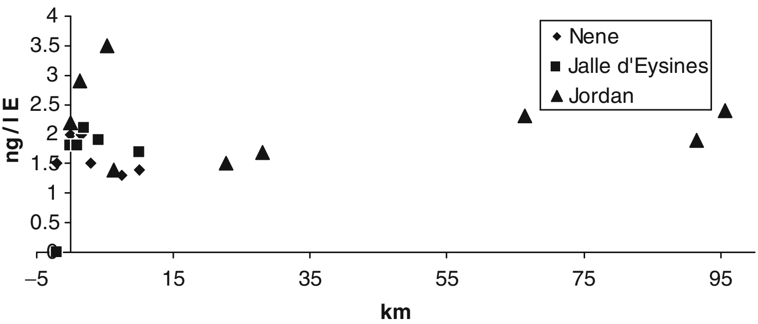


Fig. 4.3 Estrone levels in three rivers after influx of sewage water effluent during conditions of low flow (600–1,200 l/s) (based on Barel-Cohen et al. 2006; Labadie and Budzinski 2005; Williams et al. 2003). Data from the Nene and Jalle d'Elsines were above and below an SWTP at 0 km. The lower Jordan River, which does not have any fresh water sources, was measured from its origin and there were no sources of SWTPs or CAFO effluent after the 22 km mark

unrelated to the initial loading. Both estrogen and testosterone can be detected in rivers at least 60 km from their CAFO source.

4.5 Summary

Although Kolpin et al. (2002) reported that steroid hormones can be found in substantial concentrations in polluted US rivers (median values for 17α-estradiol 30 ng/l; E2 160 ng/l; estriol 19 ng/l; E1 27 ng/l; progesterone 111 ng/l; T 116 ng/l) such values are generally not seen in streams and rivers mainly receiving run-off from CAFOs (Matthiessen et al. 2006). In such streams, values for T, E and A are below 5 ng/l while estriol rapidly disappears. Biogradation is the primary route of destruction and in conditions of low bacterial activity (e.g. winter in Northern climates) and low flow, the steroids can persist for very long periods of time and be transport many km from their source. There appears to be a limit of 1–2 ng/l below which biodegradation can destroy T and E1 even where ethinylestradiol, E2 and E3 are reduced to below <0.5 ng/l. Small quantities of the steroids in the 0.1–0.3 ng/l range may be widespread in nature as the result of the steroids produced by fish or excretion from wildlife as fecal steroids are secreted by all vertebrates.

References

Barel-Cohen K, Shore LS, Shemesh M, Wenzel A, Mueller J, Kronfeld-Schor N (2006) Monitoring of natural and synthetic hormones in a polluted river. J Environ Manage 78:16–23

Finlay-Moore O, Hartel PG, Cabrera ML (2000) 17β-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J Environ Qual 29:1604–1611

Jenkins MB, Endale DM, Schomberg HH, Sharpe RR (2006) Fecal bacteria and sex hormones in soil and runoff from cropped watersheds amended with poultry litter. Sci Total Environ 358:164–177

Jürgens MD, Johnson AC, Williams RJ (1999) Fate and behavior of steroid oestrogens in rivers: a scoping study. R&D Technical Report P161. Environment Agency, London, UK

Jürgens MD, Holthaus KIE, Johnson AC, Smith JJL, Hetheridge M, Williams RJ (2002) Environ Toxicol Chem 21:480–488

Kolodziej EP, Sedlak DL (2007) Rangeland grazing as a source of steroid hormones to surface waters. Environ Sci Technol 4:3514–3520

Kolodziej EP, Harter T, Sedlak DL (2004) Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ Sci Technol 38:6377–6384

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg S, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211

Labadie P, Budzinski H (2005) Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). Environ Sci Technol 39:5113–5120

Matthiessen P, Arnold D, Johnson AC, Pepper TJ, Pottinger TG, Pulman KGT (2006) Contamination of headwater streams in the United Kingdom by oestrogenic hormones from livestock farms. Sci Total Environ 367:616–630

Nichols DJ, Daniel TC, Moore PA, Edwards DR, Pote DH (1997) Runoff of estrogenic hormone 17β-estradiol from poultry litter applied to pasture. J Environ Qual 26:1002–1006

Nichols DJ, Daniel TC, Edwards DR, Moore PA, Pote DH (1998) Use of grass filter strips to reduce 17β-estradiol in runoff from fescue-applied poultry litter. J Soil Water Conserv 53:74–77

Shore LS, Correll D, Chakroborty PK (1995) Fertilization of fields with chicken manure is a source of estrogens in small streams. In: Steele K (ed), Animal waste and the land-water interface. Lewis, Boca Raton, FL, pp 49–56

Shore LS, Reichman O, Shemesh M, Wenzel A, Litaor M (2004) Washout of accumulated testosterone in a watershed. Sci Total Environ 332:193–202

Williams RJ, Johnson AC, Smith JJL, Kanda R (2003) Steroid estrogens profiles along river stretches arising from sewage treatment works discharges. Environ Sci Technol 37:1744–1750

Chapter 5
Physiochemical Characterization of Steroid Hormones in Soil

Francis X.M. Casey

Abstract Laboratory studies on the fate and transport of reproductive, steroidal hormones and their primary metabolites indicate that aqueous concentrations are short-lived, where dissipation half-lives are on the order of a few hours or days. Dissipation of hormones is caused by i) binding or sorption to the soil and ii) degradation. Sorption of both androgenic and estrogenic hormones occurs predominantly in the organic fraction of the soil and is very rapid through time. Hormone degradation is controlled predominantly by the biological activity of the soil. Hormones will persist in soil where the biological activity is low, such as sterilized or cool low-oxygen soils. Laboratory soil column studies have also indicated that mobility of androgenic and estrogenic hormones is limited, where little will escape the top 5 cm of soil.

5.1 Physiochemical Characteristics of Steroids in Soil

Once manures are applied to the land, soil processes will largely determine the fate of hormones. The water solubilities of E2 and T are 13 (Lai et al. 2000) and 18–25 (Sugaya et al. 2002) mg L^{-1} at 20°C, and their octanol–water partition coefficients are 3.94 (Lai et al. 2000) and 3.22 (Núñez and Yalkowsky 1997) respectively. Both E1 and androstenedione are slightly more soluble than the parent compound (Kim et al. 2007; Yu et al. 2004). These compounds are hydrophobic and their mobility should be limited in soils. When natural steroidal hormones are released into the environment, they undergo several fate and transport processes that control degradation, sorption, and mobility, which have been identified by various researchers (Casey et al. 2003, 2004, 2005; Das et al. 2004; Holthaus et al. 2002; Jacobsen et al. 2005; Lai et al. 2000; Layton et al. 2000; Lee et al. 2003; Mansell and Drewes

F.X.M. Casey (✉)
North Dakota State University, 123 Walster Hall, Fargo, ND, 58105, USA
e-mail: francis.casey@ndsu.edu

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_5,

2004; Schicksnus and Müller-Goymann 2000; Yu et al. 2004). Natural hormone degradation rates are rapid (0.060–0.134 h^{-1}) (Fan et al. 2007a,b) even at part-per trillion concentrations (Colucci and Topp 2002). Also, degradation readily occurs under aerobic but not anaerobic conditions (Fan et al. 2007a,b; Joss et al. 2004), is facilitated under warmer temperatures (Hemmings and Hartel 2006; Jacobsen et al. 2005), and is correlated to water content (Colucci et al. 2001). Bioavailability plays a large role in the rate of hormone degradation (Fan et al. 2008; Fan et al. 2007a; Jacobsen et al. 2005), which is related to sorption. Abiotic mechanisms, such as photodegradation, can also degrade steroidal hormones (Fan et al. 2007b; Jürgens et al. 2002; Mansell et al. 2004).

5.2 Absorption and Deabsorption

The primary sorption domain of steroidal hormones is organic carbon, and partitioning is consistent with the hydrophobic process (i.e., there is a linear relation between the Log_{10} octanol–water portioning coefficient ($\mathrm{Log}\,K_{ow}$) and $\mathrm{Log}\,K_{oc}$ [$\mathrm{Log}\,K_{oc} = \mathrm{Log}_{10}(K_d/(OC/100))$; where K_d is the linear partitioning coefficient [$\mathrm{L}\,\mu\mathrm{g}^{-1}$] and OC is the fraction of organic carbon]) (Lee et al. 2003). The hydrophobic partitioning of hormones would explain sorption correlations with soil particle sizes and organic matter content (Casey et al. 2003, 2005; Das et al. 2004; Lai et al. 2000; Lee et al. 2003; Yu et al. 2004). Sorption has also been shown to have a kinetic component that is related to the soil organic content (Casey et al. 2003, 2005; Das et al. 2004; Holthaus et al. 2002; Lai et al. 2000). Additionally, non-hydrophobic sorption interactions may occur, where it is hypothesized that the phenolic group of 17β-estradiol and estrone interact with humic acids or mineral surfaces via hydrogen and covalent bonding (Yu et al. 2004). Two recent studies (Fan et al. 2007a,b) have shown, through solvent extraction (Kaplan and Kaplan 1982) that ^{14}C labeled E2 and T will become irreversibly sorbed into various organic matter fractions (e.g. humic acid, fulvic acid, and humin). These soil fate and transport studies indicate that there should be limited detections in the environment, due to the labile nature of these compounds; nonetheless, natural hormones are consistently detected at part-per trillion levels in the environment (Barber et al. 2000; Kolpin et al. 2002).

5.3 Sorption, Transformation and Mobility: Laboratory Experiments

Table 5.1 provides some properties of the soils used for the laboratory experiments reported by Casey et al. (2003, 2004). Table 5.2 shows the calculated sorption coefficients normalized to the organic carbon content and the sorption rate constants.

The 17β-estradiol sorbs stronger and faster to the soil compared to testosterone, but both are bound relatively strongly to the soil as indicated by the $\log K_{oc}$ values (Table 5.1).

Sorption affinities between soil fractions with both 17β-estradiol and testosterone are evident. Hormone bound to the soil is correlated to %clay and organic carbon fraction, and inversely correlated to %sand (Casey et al. 2003, 2004). Essentially, these correlations are indications that both of these compounds are non-polar and hydrophobic, so they associate with the organic fraction of the soil. Casey et al. (2005) showed a linear relation between the $\mathrm{Log}\,K_{oc}$ and octanol–water partitioning coefficient for 17β-estradiol and estrone, which demonstrated the hydrophobic partitioning process of these compounds.

The soil column experiments from Casey et al. (2003, 2004, 2005) indicate that both parent 17β-estradiol and testosterone will have limited mobility in a variety of soil types (Table 5.1). Even intact soil, where soil columns were harvested from fields and include structure, cracks, etc., showed little mobility of parent estrogenic and androgenic compounds in a Hecla-Hamar soil (Table 5.1; Fan et al. 2007a; Fan et al. 2008). Fan et al. 2008 indicated that only 6% of total applied ^{14}C mass from radiolabelled 17β-estradiol was collected in the effluent of a 25 cm long column. Of this effluent, none was parent hormone (i.e., 17β-estradiol), 1.5% was estrone, and 4.2% was an unidentified polar metabolite. Inside the column, approximately 70% of the ^{14}C was recovered; where nearly 50% was non-extractable (i.e., it was recovered through combusting the soil) and 23% was extractable. Moreover, 50% of the total applied 17β-estradiol was recovered in the top 5 cm of soil. Finally, the extractable ^{14}C from inside the column was composed of <2% 17β-estradiol, 20% estrone, and 55% unidentified polar metabolite. Fan et al. (2007a) showed similar results of minimal mobility of testosterone in the same intact soil (Fig. 5.1). Fan et al. (2007a) showed that ~63% of the testosterone was recovered from the top 5 cm of the soil; and no parent hormone was recovered in the column effluent. Additionally, approximately 23% of the testosterone was converted to $^{14}CO_2$ through mineralization.

Fan et al. (2007b) compared the fate of 17β-estradiol and testosterone in sterilized and natural soil under aerobic and anaerobic conditions. This comparison first indicates that the fates of these hormones are largely controlled by the biology of the soil–water system, where far less mineralization and transformations occur under sterile conditions. For testosterone, under aerobic conditions, 83% of the soil extract was metabolite; whereas, 87% of the extract was parent testosterone in the sterile soil. For 17β-estradiol, under aerobic conditions, 84% of the soil extract was metabolite; whereas, 73% of the extract was parent 17β-estradiol in the sterile soil. Secondly, there are large differences in the mineralization of testosterone compared to 17β-estradiol. Six percent of 17β-estradiol was mineralized to $^{14}CO_2$ in natural soils under aerobic conditions, compared to about 63% of the testosterone in 120 h (Table 5.3; Fan et al. 2007b). Under anaerobic conditions only 0.9% of the 17β-estradiol was mineralized compared to 46% for testosterone.

Table 5.1 Soil types and properties of laboratory experiments from Casey et al. (2003, 2004)

Soil series	Taxonomic description	Organic matter content (%)	Specific surface area[a] ($m^2\,g^{-1}$)	pH	Texture (sand:silt:clay)
Bearden – silty clay loam	Fine-silty, mixed, superactive, frigid Aeric Calciaquoll	7.5	175	6.4	15:51:34
Gardena – clay loam	Coarse-silty, mixed, superactive, frigid Pachic Hapludoll	5.3	154	6.4	29:44:27
Glyndon – sandy clay loam	Coarse-silty, mixed, superactive, frigid Aeric Calciaquoll	3.3	123	7.9	52:27:21
LaDelle – silt loam	Fine-silty, mixed, superactive, frigid Cumulic Hapludoll	9.2	151	7.3	12:62:26
Sioux – loam	Sandy-skeletal, mixed, frigid Entic Hapludolls	7.5	106	7.8	46:34:21
Sand	–	0.0	–	n/a	100:0:0
Hecla-Hamar – loamy fine sand	Sandy, mixed, frigid Oxyaquic Hapludolls	2.2	–	6.9	14:19:67

[a]Specific surface area indicates the surface area of a porous media contained in unit mass and is defined as: specific surface area = (surface area)/(mass of soil)

Table 5.2 Organic-content normalized partition coefficients (Log K_{oc}) and the calculated first-order degradation rate constants (μ) determined for 17β-estradiol and testosterone following miscible-displacement experiments in various soils (from Casey et al. 2003, 2004)

	17β-Estradiol		Testosterone	
Soil type	Log K_{oc}	μ (h^{-1})	Log K_{oc}	μ (h^{-1})
Bearden – silt clay loam	6.02	1.494	3.30	0.404
Gardena – clay loam	6.07	2.800	2.97	0.496
Glyndon – sandy clay loam	7.18	0	4.56	0.600
LaDelle – silt loam	5.85	0.480	3.03	0.493
Sioux – loam	5.94	0.284	3.07	0.493

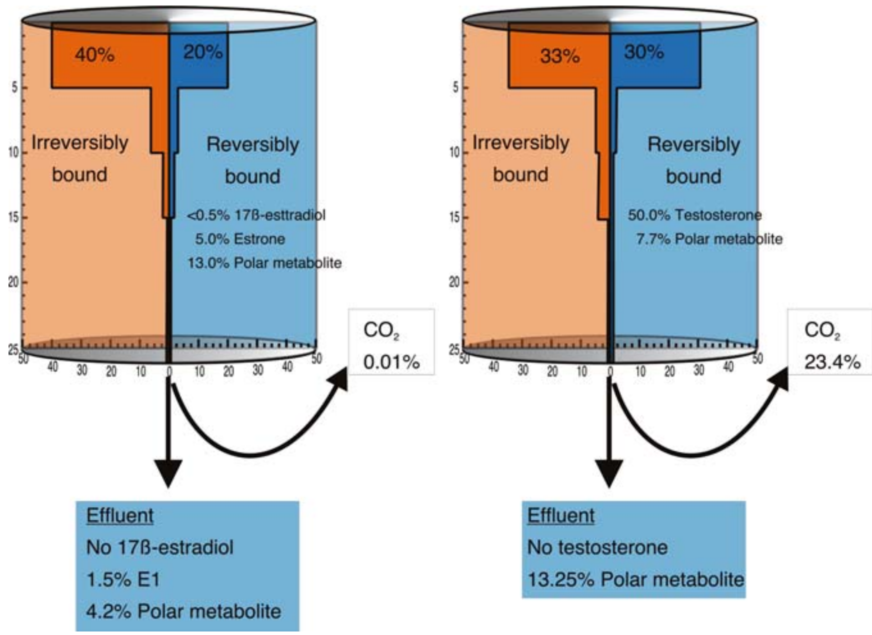


Fig. 5.1 The distribution of ^{14}C labeled 17β-estradiol (*left*) and testosterone (*right*) in soil column transport experiments from Fan et al. (2008) and Fan et al. (2007a), respectively

The laboratory results indicate that testosterone and 17β-estradiol are rapidly dissipated with half-lives typically ranging from a few hours to a few days in a range of soils incubated under a range of temperature and moisture conditions. Further research supporting this general conclusion was conducted by Lee et al. (2003), Colucci et al. (2001), Colucci and Topp (2002), Das et al. (2004) and Lorenzen et al. (2005).

Table 5.3 17β-Estradiol and testosterone recoveries from natural (non-sterile) and autoclaved (sterile) soils under aerobic and anaerobic conditions. The recoveries are broken down by gas trapped, extractable (water and acetone), and non-extractable. The non-extractable was characterized for its association with different soil organic fractions (from Fan et al. 2007b)

	Fraction of applied dose recovered			
	Aerobic		Anaerobic	
	Natural	Autoclaved	Natural	Autoclaved
	%Dose		%Dose	
17β-Estradiol				
Trapped ^{14}C				
$^{14}CO_2$	6	0.2	0.9	0
$^{14}CH_4$	0	0	0	0
Extractable ^{14}C				
H_2O	2	2	2	1
Acetone	10	26	17	23
Subtotal	12	28	19	24
Non-extractable				
Humic acids	37	31	37	24
Fulvic acids	17	13	22	15
Humus	19	23	11	11
Subtotal	73	67	70	50
Total	91	95	90	74
Testosterone				
Trapped ^{14}C				
$^{14}CO_2$	63	0.2	46	0
$^{14}CH_4$	0	0	2	0
Extractable ^{14}C				
H_2O	0.4	0.2	0	0.5
Acetone	3	25	16	37
Subtotal	3.4	25.2	16	37.5
Non-extractable				
Humic acids	3	11	5	9
Fulvic acids	9	2	0	3
Humus	7	41	20	37
Subtotal	19	54	25	49
Total	85	79	89	87

References

Barber LS, Brown GK, Kolpin DW, Writer JH, Zaugg SD (2000) A reconnaissance for hormone compounds in the surface waters of the United States, p. 48, In: Wilde FD, Britton LJ, Miller CV, Koplin DW (eds) Effects of animal feeding operations on water resources and the environment. Proceedings of the technical meeting, Fort Collins, CO, Aug 30–Sept 1, 1999, U.S. Geological Survey Open-File Report 00-204. U.S. Geological Survey, Reston, VA, p 48

Casey FXM, Larsen GL, Hakk HH, Šimůnek J (2003) Fate and transport of 17β-estradiol in soil-water systems. Environ Sci Technol 37:2400–2409

Casey FXM, Hakk H, Šimůnek J, Larsen GL (2004). Fate and transport of testosterone in agricultural soils. Environ Sci Technol 38:790–798
Casey FXM, Šimůnek J, Lee J, Hakk H, Larsen GL (2005) Sorption, mobility, and transformation of estrogenic hormones in natural soil. J Environ Qual 34:1372–1379
Colucci MS, Topp E (2002) Dissipation of part-per-trillion concentrations of estrogenic hormones from agricultural soils. Can J Soil Sci 82:335–340
Colucci MS, Bork H, Topp E (2001) Persistence of estrogenic hormones in agricultural soils: I. 17β-Estradiol and estrone. J Environ Qual 30:2070–2076
Das BS, Lee LS, Rao PSC, Hultgren RP (2004). Sorption and degradation of steroid hormones in soils during transport column studies and model evaluation. Environ Sci Technol 38:1460–1470
Fan Z, Casey FXM, Hakk H, Larsen GL (2008). Modeling coupled degradation, sorption, and transport of 17β-estradiol in undisturbed soil. Water Resour Res 44:W08424, DOI 10.1029/2007WR006407
Fan Z, Casey FXM, Larsen GL, Hakk H (2007a) Persistence and fate of 17β-estradiol and testosterone in agricultural soils. Chemosphere 67:886–895
Fan Z, Casey FXM, Hakk H, Larsen GL (2007b) Discerning the fate and transport of testosterone in undisturbed soil: model development and experimental evaluation. J Environ Qual 36:864–873
Hemmings SNJ, Hartel PG (2006) Mineralization of hormones in breeder and broiler litters at different water potentials and temperatures. J Environ Qual 35:701–706
Holthaus KIE, Johnson AC, Jurgens MD, Williams RJ, Smith JJL, Carter JE (2002) The potential for estradiol and ethinylestradiol to sorb to suspended and bed sediments in some English rivers. Environ Toxicol Chem 21:2526–2535
Jacobsen A, Lorenzen A, Chapman R, Topp E (2005) Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids. J Environ Qual 34:861–871
Joss A, Andersen H, Ternes T, Richle PR, Siegrist H (2004) Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ Sci Technol 38:3047–3055
Jürgens MD, Holthaus KIE, Johnson AC, Smith JJL, Hetheridge M, Williams RJ (2002) Environ Toxicol Chem 21:480–488
Kaplan DL, Kaplan AM (1982) Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Appl Environ Microbiol 35:949–954
Kim I, Yu Z, Xiao B, Huang W (2007) Sorption of male hormones by soils and sediments. Environ Toxicol Chem 26:264–270
Koplin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211
Lai KL, Johnson LK, Scrimshaw MD, Lester JN (2000) Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ Sci Technol 34:3890–3894
Layton AC, Gregory BW, Seward JR, Schultz TW, Sayler GS (2000) Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee U.S.A. Environ Sci Technol 34:3925–3931
Lee S, Strock TJ, Sarmah AK, Rao PSC (2003) Sorption and dissipation of testosterone, and estrogens, and their primary transformation products in soils and sediments. Environ Sci Technol 37:4098–4105
Lorenzen A, Chapman R, Hendel JG, Topp E (2005) Persistence and pathways of testosterone dissipation in agricultural soil. J Environ Qual 34:854–860
Mansell J, Drewes JE (2004) Fate of steroidal hormones during soil-aquifer treatment. Ground Water Monit Remed 24:94–101
Mansell J, Drewes JE, Rauch T (2004) Removal mechanisms of endocrine disrupting compounds (steroids) during soil aquifer treatment. Water Sci Technol 50:229–237
Núñez FAA, Yalkowsky SH (1997) Correlation between log *P* and ClogP for some steroids. J Pharm Sci 86:1187–1189

Schicksnus T, Müller-Goymann CC (2000) 17β-Estradiol solubility in aqueous systems – influence of ionic strength, pH and adsorption to packaging material. Arch Pharm Pharm Med Chem 333:66

Sugaya Y, Yoshiba T, Kajima T, Ishihama Y (2002) Development of solubility screening methods in drug discovery. Yakugaku Zasshi 122:237–246

Yu Z, Xiao B, Huang W, Peng P (2004) Sorption of steroid estrogens to soils and sediments. Environ Toxicol Chem 23:531–539

Chapter 6
Transport of Steroids in Soil Under Field Conditions

Laurence S. Shore and Francis X.M. Casey

Abstract Although the behavior of soils in batch experiments has been extensively described, there is little field work on actual soils. After amendment with animal manures, nearly all of the estrogen is found bound to the topsoil. In contrast, testosterone readily penetrates the vadose zone and reaches the groundwater. However, in some situations like water saturated soils or karst formation, estrogen also can reach the groundwater.

6.1 Introduction

The extraction and identification of steroids in soils under laboratory conditions have been described in the previous chapter. Under field conditions, results can be much more varied. Agricultural soils are generally a mixture of silt, clay and sand. The topmost layer is the topsoil usually about 15–20 cm. The rhizosphere is the area of the topsoil surrounding the roots where most of the bacterial activity takes place. The area from the topsoil to the saturated zone or water table is the unsaturated zone or the vadose zone. The transition zone from the vadose zone to the saturated zone is called the capillary layer. Water tables may be shallow, less than 10 m, or deeper (10–40 m). As a rule of thumb, organic compounds descend through soils at a rate of 0.5–1.5 m/year. However, many soils display preferential flow, e.g. fissures in the soil or macropores. Karst is a collective term referring to geographic locales or geologic terrains that are dominated by limestone that has been altered through dissolution to create secondary porosity and permeability in the rock. Karst generally consist of limestone, but may be formed of other soluble rocks as well.

L.S. Shore and F.X.M. Casey (✉)
North Dakota State University, 123 Walster Hall, Fargo, ND, 58105, USA
e-mail: francis.casey@ndsu.edu

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_6, © Springer Science+Business Media, LLC 2009

6.2 Groundwater Under a Farm Raising Chickens and Pigs

At a site in Ephata PA, approximately 110,000 chickens (five sets of 22,000) and 1,500 hogs were raised each year from 1985–1993. Most manure produced at the farm animal production facility was applied to cropped acreage at the site. The farmer at the Ephrata site reported applying approximately 72,500 kg of wet poultry manure/litter during each of 1991–1992 which were typical years of broiler and crop production. This meant that approximately 1.8 g of E and 6.0 g of T were applied to the fields at the Pennsylvania farm each year. Wells were drilled to delimit the water boundaries of the aquifer under the farm at 14–30 m. It was calculated that 20–40 mg of T/year crossed the boundaries under the farm. This would represent about 1.5% of annual load. Estrogen was undetectable (<0.5 ng/L) in the groundwater and therefore less than 0.5% of the estrogen reached the groundwater. In the stream receiving the groundwater from the farm, the T concentration was about 1 ng/L and the estrogen was essentially undetectable (Shore et al. 1997).

6.3 Steroids in the Vadose Zone and Groundwater in Fields Irrigated with Treated Sewage Water

E and T are present in high amounts in treated sewage water used for irrigation (up to 300 ng E/L, 700 ng T/L; Shore et al. 1992, 1993). Wells were drilled in at eight orchards in central Israel, four which had been irrigated with treated sewage water for 20 years and four in which only fresh water was used. Soil samples were taken every 0.5 m to the water table at 25 m. T and E were mostly below 1 ng/kg except a small amount noted in the capillary zone (5–7 ng/kg) and there were no differences between the treated and reference fields. Neither T nor E was present in the saturated zone (groundwater).

6.4 Steroids in the Topsoil Under Cattle Pasture

Three fields representing control (never grazed for 20 years); moderate (0.5 cow/ha); and heavy grasing (0.83 cows/ha) were sampled at surface and at 20 cm. There was a rocky layer about 3 m below the surface that prevented transport to the vadose zone and all of the water loss was essentially due to evaporation. When the control and grazed fields were compared, it was found that on the grazed fields the testosterone concentration in the surface soil and at 20 cm depth contained between 43 and 75 ng testosterone/kg while the control field had less than 10 ng/kg. Neither A nor E were detected in these samples (<5 ng/kg).

To evaluate a field with runoff and high water table (<0.5 m), a fenced field in which beef cattle were not present most of the year with a pool receiving runoff

Table 6.1 Testosterone and androstenedione extracted from soil samples at 5, 15, and 30 cm in a field use for cattle pasture, 4, 5 and 6 months after introduction of the cattle

Soil depth	4 months	5 months	6 months
Testosterone (ng/kg)			
5 cm	280	250	228
15 cm	130	86	56
30 cm	124	182	40
Androstenedione (ng/kg)			
5 cm	26	50	28
15 cm	BLD	40	BLD
30 cm	BLD	BLD	BLD

Each value represents a single determination done in duplicate
BLD below limit of detection of 20 ng/kg

from the field was studied after the introduction of the cattle for 3 months. The grazing intensity was 0.63 cows/ha. Soil samples were taken for the first 30 cm. E was undetectable and A was present only in the upper 5 cm. T was present in all samples to 30 cm (Table 6.1).

6.5 Steroids in the Topsoil After Application of Chicken Litter

After application of chicken litter at 3.1 E2 and 0.09 T mg/ha, the average concentration of testosterone in the upper 5 cm of soil ranged from 1.1 to 72 ng/kg, and the average concentration of estradiol ranged from 65 to 636 ng/kg (Jenkins et al. 2006). Finlay-Moore et al. (2000) found T concentrations in the upper 2.5 cm were higher in moderately grazed plots (165 ng/kg) compared with the amended crop land (118 ng/kg). Corresponding concentrations for estradiol were 164 ng/kg in grazed plots and 128 ng/kg in hayed plots. Prior to the application of litter, the concentrations were about 65 ng/kg soil for both T and E. The authors did not have an explanation for the high levels of hormones in the unamended soil.

6.6 Steroids in the Vadose Zone Under a Dairy Farm Cattle Lagoon

To study the transport of the steroids in the vadose zone over long time exposure (30 years), a dairy barn with 50 dairy cattle was studied (Arnon et al. 2008). This was calculated as an annual load of 36 kg estrogens and 7 kg androgens. All of the effluent was released into a 15×10 m lagoon, which drained toward a dry creek, creating a constant overflow of about 300 m long and 1–4 m wide. The lithology under the waste lagoon consists of 8 m of clay layer on top of sand and calcareous formation and the water table was at a depth of 47 m. Soil samples (5 g) were

	Estrogen	Testosterone	Androstenedione
Input into lagoon	250 mg/dy	5 mg/dy	5 mg/dy
Clay 7 meters	800 ng/kg	700 ng/kg	50-100 ng/kg
Sand 23 meters	100 ng/kg	90 ng/kg	<50 ng/kg
Groundwater	3 ng/l	3 ng/l	<0.5 ng/l

Fig. 6.1 Schematic representation of estrogen (E1 + E2), testosterone and androstenedione in soil underneath a dairy lagoon receiving effluent from dairy barn of 50 cows for 40 years. The clay was saturated so there was little aerobic activity. The sandy layer was about 25% saturated

extracted with organic solvents and analyzed using radioimmunoassay or ELISA. Groundwater samples (500 ml; after pumping four well volumes) were extracted on C-18 solid extract columns and similarly analyzed. It was found that testosterone was present (>50 ng/kg) from the top soil throughout the vadose zone to the water table. Estrogen was above 50 ng/kg to a depth of 25 m (range 50–100 ng/kg) and androstenedione was present to 5 m. Testosterone and estrogen, but not androstenedione, were present in the groundwater (3 ng/L) (Fig. 6.1). The level of the steroids in control soils and groundwater taken 1 km upgradient from the site were below the limit of detection with the exception of one topsoil sample (2 cm; 80 ng/g). Multiple simulations using HYDRUS, a model for transport of solutes in soil based on physiochemical characteristics and absorption kinetics, could not account for the distribution of estrogen or testosterone in the soil. The authors (Arnon et al. 2008) concluded that factors other than physiochemical properties of the compounds were responsible for the observed distribution, involving preferential flow paths or interactions between hormones and manure (Stumpe and Marschner 2007; Schiffer et al. 2004). In addition, as suggested by data from SWTPs (Joss et al. 2004) and soil column experiments (Fan et al. 2007), under anaerobic conditions, such as under the lagoon, the steroids would be expected to be poorly metabolized.

6.7 Steroids in Subsurface Water Under Swine Facilities and Fields Amended with Swine Slurry

6.7.1 Site Description

Field observations of hormone concentrations were monitored at a swine operation (~4,000 swine at any one time) that included nursery and finishing facilities. The predominant soil at this farm is a Hecla-Hamar loamy fine sand (sandy, mixed, frigid oxyaquic hapludolls) developed from glacial outwash and is 67% sand (Thompson et al. 2009).

Pigs were received at this location at about 17 days old and placed in the nursery (6–10 weeks). They were then transferred into hoop or finishing barns, where they were kept until they achieved a market weight (~250 lbs). These finishing barns were uninsulated structures built on compacted earth that is covered with a bedding of straw or wood shavings. The animals ate on one side of these elongated barns and deposited their manure on the other side. The bedding/manure mixture in each hoop barn was scraped and replaced twice a year. The scraped manure/bedding mixture was piled uncovered on the bare soil, and decompose under near anaerobic conditions until they were spread on row-cropped fields (~1 year later).

6.7.2 *Hormones in Soil Water Measured with Lysimeters at 60 cm Under a Swine Facility*

Between 2003 and 2005, the Manure Storage Pond (MSP), manure, raw hoop barn manure, and the manure pile were characterized for hormones. Using LC-MS-MS, the following E2 concentration ranges were measured: raw hoop barn manure = 2.989–7.744 μg/kg, static manure pile mixture = 1.016–5.820 μg/kg, and MSP = 0.509–3.767 μg/L. For T: raw manure = 0–0.1014 μg/kg, static manure pile mixture = 0 μg/kg, and MSP = 0–0.245 μg/L.

A passive capillary lysimeter (Boll et al. 1992; Holder et al. 1991) was placed 60 cm under the uncovered manure pile described above. Another lysimeter was placed 60 cm below a hoop barn in a location where the swine excrete their manure. Also, wells were placed in the shallow oxidized aquifer (~2 m) down groundwater gradient from potential hormone sources (e.g., hoop barn, static manure pile, MSP). There were no detections of hormones in the lysimeter under the hoop barn, because the barn was covered, which blocked any precipitation and thus prevented substantial fluxes of water that could advectively transport hormones. Lysimeter water collected beneath the static manure pile contained E2 concentrations that ranged from 0–2.921 μg/L (n = 22; detects = 10; mean = 0.144 μg/L), which were nearly the same as concentrations found in the MSP. Thompson (2005) also observed that the cumulative mass recovered of 17β-estradiol in the lysimeters was approximately 150–270, 50–475, 100–425, and 225–280 ng for the MSP, manure, compost, and control treatments, respectively. These mass recoveries were 2–69%, 440–1110%, and 129–824% of the manure-borne 17β-estradiol applied on the MSP, manure, and compost, respectively.

Between 2003–2005, E2 concentrations from all the shallow wells (~2 m) ranged from 0–0.1038 μg/L (n = 206; detects = 79; mean = 0.0099 μg/L), and the T concentrations ranged from 0–0.0765 μg/L (n = 109; detects = 72; mean = 0.0023 μg/L). There were no apparent spatial associations of the hormone detections to potential hormone sources. The concentrations of T were lower than E2 in both the wells and lysimeters, but T was detected more frequently than E2 in the wells. The well detection frequency for T and E2 was 0.66 and 0.38, respectively, which may reflect T's greater mobility in the soil (Casey et al. 2003, 2004).

6.7.3 Hormones in Subsoil Measured with Lysimeters at 60 cm Under Fields Amended with Swine Manure

The concentrations of land-applied, manure-borne hormones in the subsoil was investigated from various manure types (Thompson 2005). Different manure types were applied to four small corn plots (~2 × 2 m) and two Lysimeter (60 cm deep) were placed under each plot. The manure treatments on the plots were (i) MSP liquid manure, (ii) raw manure (from the hoop barns), (iii) manure pile, and (iv) control (no manure application). Manure was applied according to university recommendations for soil nutrient requirements (Schmitt 1999) and these treatments were constant for 3 years. The research plots were close together, in order to minimize disruption of normal farm operations. A suite of four non-sorbing, conservative fluorobenzoic acid tracers were also used to identify whether there was any cross-migration from one plot to another and to identify residence times through the soil (Casey et al. 1997). The 3 years of continuous (except winter) subsurface water quality is summarized in Table 6.2. There were no identifiable interactions between the lysimeters based on tracer data. Also, hormone detections were greater during cooler and wetter weather, normally in the spring. Furthermore, the mass of hormones collected in the lysimeters was higher than expected based on laboratory experiments (Casey et al. 2003, 2004, 2005; Fan et al. 2007) and may reflect contributions from other sources. The high hormone recovery may also represent antecedent concentrations that are naturally present in the environment. These lysimeter concentrations are also consistent with those reported by Herman and Mills (2003).

6.8 Steroid Transport in Karst Formations

Water in springs from a mantled karst aquifer recharged after rain events contained levels of 6–66 ng/L of E2 and the estradiol concentration correlated with the *E. coli* and fecal coliform counts (Peterson et al. 2000). Under conditions of cold and darkness typical of cave streamwater (Peterson et al. 2005), the E2 was stable and did not decrease unless exposed to ambient temperature and light. Therefore, although E2 usually rapidly disappears in soils, it is possible limestone formations with preferential flow do not reduce the E2 to E1.

Table 6.2 Three-year summary of lysimeter water quality data beneath plots fertilized with manure from a manure storage pond (MSP), manure, static pile, or no treatment (control)

	MSP	Manure17β-estradiol	Static pile	Control	MSP	Manure Testosterone	Static pile	Control
Samples (detections)	51 (29)	41 (26)	46 (19)	46 (26)	30 (10)	17 (8)	24 (12)	22 (11)
Avg. conc. (μg/L)	0.011	0.011	0.011	0.019	0.003	0.004	0.002	0.002
Stdev. conc. (μg/L)	0.015	0.012	0.037	0.079	0.007	0.010	0.002	0.002

6.9 Comparison of Field Studies to Laboratory Studies

The laboratory studies described in Chap. 5 indicated that both 17β-estradiol and testosterone are expected to dissipate on the order of hours to few days through either the process of degradation or sorption in soil. However the subsurface field observations indicated that both 17β-estradiol and testosterone had greater mobility and persistence compared to laboratory results (Thompson 2005; Casey et al. 2008).

As described in Sect. 6.7, Thompson (2005) installed eight passive capillary lysimeters 60 cm below soil surface and collected soil water percolations beneath plots fertilized with hog manure. The field soils of the Thompson (2005) study were identical to Hecla-Hamar soil (Table 5.1) used by (Fan et al. 2007, 2008) in their laboratory studies. Manure was applied to three plots at rates according to soil nutrient needs, and a fourth plot was control, or no applied manure. Thompson (2005) also applied a non-sorbing, conservative (non-degrading) trace to each plot to identify whether cross-migration from plot-to-plot occurred, and to identify rate of transport from the soil surface. Greater than expected transport of 17β-estradiol occurred in the plots, where 17β-estradiol was mobile with the percolating water, and it appeared in the lysimeter effluent at the same time or even earlier than the surface applied non-sorbing tracer. As can be seen in Table 6.2, no distinctions of hormone concentrations or detections between the manure treatments or even between manure treatments and control were observed.

In this on-farm study, the early arrival of 17β-estradiol in the lysimeter effluent and the greater mass recoveries indicate that hormone was antecedently present in the soil profile. Additionally, this demonstrates that hormone persistence and mobility is facilitated by some other factor.

Casey et al. (2008) reported a field lysimeter transport experiment, where dissolved hormone was deliberately applied to the soil surface and concentrations were measured throughout the soil profile and in the lysimeter effluent (2.3 m deep). Additionally, a conservative, non-sorbing tracer was applied to this lysimeter at the same time as the hormone. The lysimeter was constructed in 1980 (20 years prior to the hormone transport experiment), in a Hecla soil, which is very similar to the Hecla-Hamar in Table 5.1. Tracer, 17β-estradiol, and testosterone concentrations were detected in the effluent and throughout the soil profile.

Statistical analysis of the hormone concentrations indicated that soil-water status and organic matter content in the soil profile were significant in explaining both 17β-estradiol and testosterone concentrations. Both 17β-estradiol and testosterone were significantly related in an inverse manner to percent saturation. This indicates the greater persistence of hormone under low oxygen conditions, which corresponds to the results of Fan et al. (2007). The significance of organic matter content may indicate the importance of hydrophobic sorption in the fate of hormone (e.g., Casey et al. 2005).

Again, compared to laboratory results, Casey et al. (2008) found greater than expected mobility of hormones in the field lysimeter; where hormone was detected almost instantaneously in the lysimeter effluent 2.3 m below surface. This is potentially explained by several factors: (i) the antecedent presence of 17β-estradiol and testosterone, (ii) analytical non-specificity of the immunoassay, and/or (iii) facilitated transport (likely colloidal; Liu et al. 2005). Casey et al. (2008) also found that 17β-estradiol and testosterone concentrations were correlated to lysimeter drainage, perhaps further indicating the significance of colloidal facilitated transport. Preferential flow was not likely a factor explaining the rapid transport of these hormones because the non-sorbing tracer was transported in a manner not consistent with flow through cracks, macropores, or other bypass flow mechanisms. Finally, colloidally bound hormone would cause the hormone to persist because it would not be biologically available. Specifically, the hormone needs to be unbound or dissolved in an aqueous solution for it to be available for microbes to convert to other metabolites or to be mineralized (Fan et al. 2007, 2008). The persistence would allow these compounds to remain unchanged in the soil profile bound to colloids. However, when near saturated water flow is induced they would be mobilized and detected.

6.10 Summary

In general, laboratory results indicate very limited persistence and mobility of hormones, while field observations suggest moderate persistence and transport. Extended persistence of hormones in the field and processes such as facilitated transport (Schiffer et al. 2004; Arnon et al. 2008; Stumpe and Marschner 2007) can result in greater than expect detections. Furthermore, although soil column experiments would indicate that transport of T should not differ greatly from E, T was consistently observed in surface and groundwater where E was absent although the relative loads of the hormones were similar (Shore et al. 1997, 2004). This is presumably the result of the phenol group of the estrogen, primarily in the form of E1, binding more strongly to the humic acids than T. Only in extreme scenarios is estrogen transported to the vadose zone or to the groundwater (6.6, 6.8). Androstenedione is intermediate between estrogen and testosterone, being partially mobile. The failure to observe A in the same soils as T is not readily explained as the chemical structure of the two compounds is almost identical (Fig. 2.1).

The antecedent presence of 17β-estradiol and testosterone also requires additional study. Even in fields that had no history of exposure to manure, values of 60–80 ng/kg of estrogen were occasionally observed in topsoil and even at 60 cm 19 ng/L estrogen was present in soil water (Arnon et al. 2008; Finlay-Moore et al. 2000; Table 6.2). The presence of steroids from unidentified sources in presumably untreated fields may be related to lack of microbial adaption in these soils not exposed to large quantities of steroids similar to the relative inability of industrial SWTPs to metabolize steroids present domestic sewage (Layton et al. 2000).

References

Arnon S, Ofer D, Elhananni S, Cohen K, Pankratov I, Gross A, et al. Transport of testosterone and estrogen from dairy farms waste lagoons to groundwater. Environ Sci Technol. 2008;42: 5521–5526

Boll J, Steenhuis TS, Selker JS. Fiberglass wicks for sampling of water and solutes in the vadose zone. Soil Sci Soc Am J. 1992;56:701–707

Casey FXM, Logsdon SD, Horton R, Jaynes DB. Immobile water content and mass exchange coefficient of a field soil. Soil Sci Soc Am J. 1997;61:1030–1036

Casey, FXM, Larsen G, Hakk HH, Šimůnek J. Fate and transport of 17β-estradiol in soil-water systems. Environ Sci Technol. 2003;37:2400–2409

Casey, FXM, Hakk H, Šimůnek J, Larsen GL. Fate and transport of testosterone in agricultural soils. Environ Sci Technol. 2004;38:790–798

Casey FXM, Šimůnek J, Lee J, Hakk H, Larsen GL. Sorption, mobility, and transformation of estrogenic hormones in natural soil. J Environ Qual. 2005;34:1372–1379

Casey FXM, Oduor P, Hakk H, Larsen GL, DeSutter TM. Transport of 17β-estradiol and testosterone in a field lysimeter. Soil Sci. 2008;173:456–467

Fan Z, Casey FXM, Hakk H, Larsen GL (2008) Modeling coupled degradation, sorption, and transport of 17β-estradiol in undisturbed soil. Water Resour. Res 44:W08424, DOI 10.1029/2007WR006407

Fan Z, Casey FXM, Larsen GL, Hakk H. Persistence and fate of 17β-estradiol and testosterone in agricultural soils. Chemosphere. 2007;67:886–895

Finlay-Moore O, Hartel PG, Cabrera ML. 17β-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J Environ Qual. 2000;29:1604–1611

Herman JS, Mills AL. Biological and hydrogeological interactions affect the persistence of 17β-estradiol in an agricultural watershed. Geobiology. 2003;1:141–151

Holder M, Brown KW, Thomas JC, Zabcik D, Murray HE. Capillary wick unsaturated zone pore water sampler. Soil Sci Soc Am J. 1991;55:1195–1202

Jenkins MB, Endale DM, Schomberg HH, Sharpe RR. Fecal bacteria and sex hormones in soil and runoff from cropped watersheds amended with poultry litter. Sci Total Environ. 2006; 358:164–177

Joss A, Andersen H, Ternes T, Richle PR, Siegrist H. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ Sci Technol. 2004;38:3047–3055

Layton AC, Gregory BW, Seward JR, Schultz TW, Sayler GS. Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee USA. Environ Sci Technol. 2000;34:3925–3931

Liu R, Wilding A, Hibberd A, Zhou JL. Partition of endocrine-disrupting chemicals between colloids and dissolved phase as determined by cross-flow ultrafiltration. Environ Sci Technol. 2005;39:2753–2761

Peterson EW, Davis RK, Orndorff HA. 17β-Estradiol as an indicator of animal waste contamination in mantled karst aquifers. J Environ Qual. 2000;29:826–834

Peterson EW, Wicks CM, Kelley CA. Persistence of 17β-estradiol in water and sediment-pore water from cave streams in central Missouri. Environ Eng Geosci. 2005;11:221–228.

Schmitt MA (1999) Manure management in Minnesota (FO-3553-GO), vol (FO-3553-GO). Minnesota Extension Service, St. Paul, MN

Schiffer B, Totsche KU, Jann S, Kögel-Knabner I, Meyer K, Meyer HHD. Mobility of the growth promoters trenbolone and melengestrol acetate in agricultural soil: column studies. Sci Total Environ. 2004;326:225–237

Shore L, Kapulnik Y, Ben-Dov B, Fridman Y, Weninger S, Shemesh M. Effects of estrone and 17β-estradiol on vegetative growth of *Medicago sativa*. Physiol Plant. 1992;84:217–222.

Shore L, Gurevich M, Shemesh M. Estrogen as an environmental pollutant. Bull Environ Contam Toxicol. 1993;51:361–366

Shore LS, Hall DW, Shemesh M. Estrogen and testosterone in ground water in the Chesapeake Bay Watershed. , Dahlia Greidinger International Symposium on Fertilization and the EnvironmentHaifa, Israel: Technion; 1997. p. 250–255

Shore LS, Reichman O, Shemesh M, Wenzel A, Litaor M. Washout of accumulated testosterone in a watershed. Sci Total Environ. 2004;332:193–202

Stumpe B, Marschner B. Long-term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17β-estradiol and testosterone in soils. Sci Total Environ. 2007;374:282–291

Thompson ML, Casey FXM, Khan E, Hakk H, Larsen GL, DeSutter TM (2009) Occurrence and pathways of manure-borne 17β-estradiol in vadose zone water. Chemosphere DOI 10.1016/j.chemosphere.2009.03.037

Chapter 7
Tracking Sources of CAFO Pollution

Laurence Shore

Abstract Defining the source of pollution is sometimes a contentious affair. Pollution from human sources is readily characterized by the presence of the synthetic estrogen, ethinylestradiol. Steroid and sterol profiles are characteristic of different CAFOs, e.g. 17α estradiol is typical of cattle, a high ratio of androstenedione/testosterone is characteristic of aquaculture. This can be combined with the new methods of bacterial phenotyping for source determination.

7.1 Introduction

A reliable method for determining of the source of fecal pollution has long been the goal of public health officials for identification of the source of pathogens, something which has gathered impetus with the increased probability of bioterrorism. In the last decade, a variety of new methods have been proposed to address the problem if the fecal contamination is from human, livestock or wildlife, the latter groups being of less concern to public health. No method by itself at the present time seems to have obtained general acceptance (Field and Samadpour, 2007; Santo Domingo et al. 2007). The methods can be summarized as bacterial or chemical. Microbial source tracing (MST) also referred to as bacteriological source tracing (BST), is based on nucleic acid sequences from bacteria presumed unique to certain species, and ribosomal types. Many of these markers require specialized laboratories and development of libraries. The main problem with MST is that it is questionable if any species specific to a existing bacterial library could be used over a large geographical area. However, global distribution of specific chicken gut flora bacteria has been demonstrated (Lu et al. 2007) so it is possible that such sequences can be found for CAFOs. Chemical substances suggested have been the fecal sterols,

L. Shore (✉)
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute,
POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_7,

fragrances and detergents (Standley et al. 2000). The usefulness of caffeine, while it is nearly universally present in human sewage, is limited by dilution and rapid destruction after secondary treatment (e.g. oxygenation ponds). The detergents provide several markers, the alkyl detergents, brightening agents, and boron. Of particular interest are the poorly biodegradable “hard” alkylphenol ethoxylates (APEOs) as they are also endocrine disruptors (Zoller et al. 2004). The pharmaceuticals used only by humans and which are persistent even after treatment in SWTPs, e.g. the synthetic estrogen, ethinylestradiol, are also possible markers (Cimenti et al. 2007). Defining CAFO contributions could then be based on the finding of steroid hormones in the absence of the synthetic steroid.

7.2 Microbial Source Testing (MST) for Defining Source of Pollution

The revolution in microbial phylogeny brought about by the discovery of 16S rDNA or ribotypes has also been applied to sourcing fecal pollution. 16S refers to the sedimentation rate in an ultracentrifuge (Svedberg units) and the rDNA means the DNA that encodes the ribosome. This method can differentiate cattle from human fecal matter (Stricker et al. 2008). Similarly classification between non-human and human fecal bacteria has been obtained using gene sequences from *E. coli* or *Bacteroides* (Ahmed et al. 2008; Call et al. 2007). More generalized methods such as the four bateriophage types differ little between cattle (types 1 and 4), swine (3 and 4) or poultry (1, 3, 4) as opposed to the clear distinction for humans (2, 3) (Sundram et al. 2006). However, Cytochrome *b* sequences from fecal mitochondrial DNA (mtDNA) can be used to differentiate cow, sheep, pig and chicken sources (Schill and Mathes 2008). Antibiotic resistance assay (ARA) can differentiate between poultry fecal matter from cattle as poultry, especially turkeys, have a very high percentage (generally >90%) of resistant flora to chlortetracycline, oxytetracyline and streptomycin while in cattle resistance is generally less than 50% (Wiggins 1996). Carbon source utilization (discussed in Chap. 8) by various *E. coli* isolates can differentiate between chicken, cattle and wildlife sources by greater than 90% (Hagedorn et al. 2003).

7.3 Chemical Source Testing

7.3.1 Coprostanol and Cholesterol

Coprostanol (5β-Cholestan-3β-ol) results from biohydrogenation of cholesterol by the gut flora (Fig. 7.1). The ratio of coprostanol to cholesterol appears to be specific to various species. Humans and pigs have more coprostanol than cholesterol while

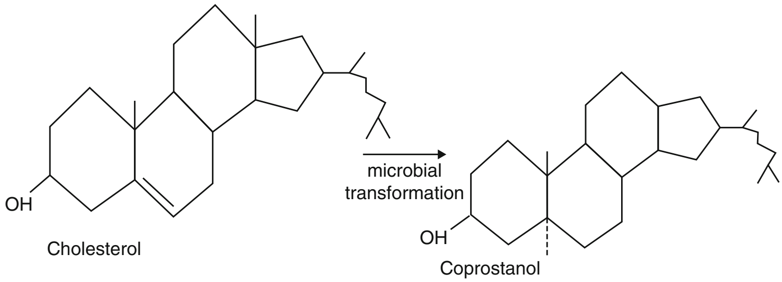


Fig. 7.1 Cholesterol conversion to coprostanol by fecal bacteria

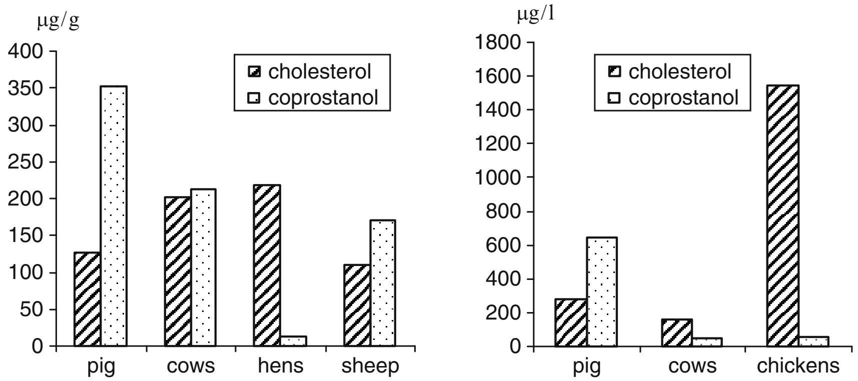


Fig. 7.2 Cholesterol and coprostanol concentrations in fecal matter (*left panel*) and in effluent from CAFOs (*right panel*). The microflora of the pig produce more coprostanol than cholesterol while ruminants about equal amounts (Leeming et al. 1996). Poultry produce very little coprostanol. These differences are also evident in the wastewater from CAFOs (Sundram et al. 2006). In humans, the ratio of coprostanol to cholesterol is similar to that of pigs

cattle have less (Leeming et al. 1996). Chickens in particular appear to produce very little coprostanol and the ratio of cholesterol to coprostanol is greater than 100 (Fig. 7.2).

7.3.2 Steroids and Ethinylestradiol

The steroids characteristic of various animal effluents have the advantage that they are easily measured and are constant over long time periods. Cattle can be identified by the high ratio of 17α-estradiol compared to other steroids; pigs by the high ratio of estriol; aquaculture by the high ratio of androstenedione to testosterone (see Chap. 2). Estrogen and testosterone are about equal in poultry fecal matter but the high coprostanol to cholesterol ratio would indicate this source. The pharmaceutical ethinylestradiol is typical of human effluent and seems to follow similar transport

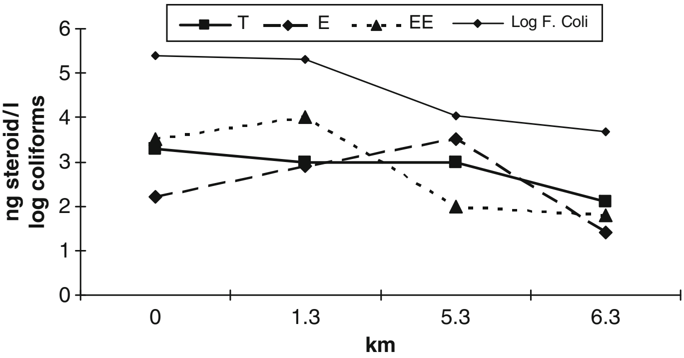


Fig. 7.3 Estrogen (E, *triangles*), testosterone (T, *squares*), ethinylestradiol (EE, *diamonds*) and log of the coliform count (log *E. coli*) over a 6 km stretch of a polluted river. The three steroids followed a similar pattern over the stretch studied (Barel-Cohen et al. 2006)

in water as the naturally occurring steroids (Fig. 7.3). However, androstenedione may be found in large amounts in effluent from pulp mills (Jenkins et al. 2003). There is no information on the environmental compartments of androsterone, a steroid unique to swine or 24-ethylepicoprostanol, a sterol unique to sheep (Leeming et al. 1996).

Pharmaceuticals unique to CAFOs (Chaps. 9 and 10) could be considered for sourcing but this would be of very limited utility as use is constantly changing due to introduction of new formulations, market conditions and new regulations.

References

Ahmed W, Stewart J, Powell D, Gardner T (2008) Evaluation of *Bacteroides* markers for the detection of human faecal pollution. Lett Appl Microbiol 46:237–242

Barel-Cohen K, Shore LS, Shemesh M, Wenzel A, Mueller J, Kronfeld-Schor N (2006) Monitoring of natural and synthetic hormones in a polluted river. J Environ Manage 78:16–23

Call DR, Satterwhite DM, Soule M (2007) Using DNA suspension arrays to identify library independent markers for bacterial source tracking. Water Res 41:3740–3746

Cimenti M, Hubberstey A, Bewtra JK, Biswas N (2007) Alternative methods in tracking sources of microbial contamination in waters. Water SA 33:183–194

Field KG, Samadpour M (2007) Fecal source tracking, the indicator paradigm, and managing water quality. Water Res 41:3517–3538

Hagedorn C, Crozier JB, Mentz KA, Booth AM, Graves AK, Nelson NJ, Reneau Jr RB (2003) Carbon source utilization profiles as a method to identify sources of faecal pollution in water. J Appl Microbiol 94:792–799

Jenkins RL, Wilson EM, Angus RA, Howell WM, Kirk M (2003) Androstenedione and progesterone in the sediment of a river receiving paper mill effluent. Toxicol Sci 73:53–59

Leeming R, Ball A, Ashbolt N, Nichols P (1996) Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res 30:2893–2900

Lu J, Domingo JS, Shanks OC (2007) Identification of chicken-specific fecal microbial sequences using a metagenomic approach. Water Res 41:3561–3574

Santo Domingo JW, Bambic DG, Edge TA, Wuertz S (2007) Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution. Water Res 41:3539–3552

Schill WB, Mathes MV (2008) Real-Time PCR detection and quantification of nine potential sources of fecal contamination by analysis of mitochondrial Cytochrome *b* targets. Environ Sci Technol 42:5229–5234

Standley LJ, Kaplan LA, Smith D (2000) Molecular tracers of organic matter sources to surface water resources. Environ Sci Technol 34:3124–3130

Stricker AR, Wilhartitz I, Farnleitner AH, Mach RL (2008) Development of a Scorpion probe-based real-time PCR for the sensitive quantification of *Bacteroides* sp. ribosomal DNA from human and cattle origin and evaluation in spring water matrices. Microbiol Res 163:140–147

Sundram A, Jumanlal N, Ehlers MM (2006) Studies to distinguish between human and animal faecal pollution using F-RNA coliphages and faecal sterols. Water SA 32:627–632

Wiggins BA (1996) Discriminant analysis of antibiotic resistance patterns in fecal *Streptococci*, a method to differentiate human and animal sources of fecal pollution in natural waters. Appl Environ Microbiol 621:3997–4002

Zoller U, Plaut I, Hushan M (2004) The case of the nonionic alkylphenol ethoxylates in the Mediterranean Sea region: is there a problem? Water Sci Technol 50:79–84

Chapter 8
Soil Ecology and Factors Affecting Biomass

Yosef Steinberger and Laurence Shore

Abstract Effluents containing pharmaceuticals and hormones from CAFOs are of concern because they are directly applied to the soil without any pre-treatment. To evaluate the potential affect of these compounds, an understanding of soil ecology is required. Soil ecology is a daunting topic as there is so much soil mass and diversity in the world. However, some parameters are consistent for the study of the factors of soil ecology, like nematode numbers and bacterial/fungal biomass. The biomass can by characterized by using arrays of distinct substrates used or amounts of specific chemicals produced, e.g. ergosterol is produced primarily by fungi.

8.1 Introduction

The increase in global population has precipitated an exponential use of natural resources with substantial amounts of synthetic compounds reaching the environment. Well over 90,000 synthetic chemicals are used on a daily basis by pharmaceutical, food and chemical companies as well as in agriculture. However, only a few of these putative toxic agents have been evaluated as to their potential for causing deleterious health and/or environmental effects. In order to be able to measure their effects on human made and natural ecosystems, specific measurable effects on scientific paradigms are required. The International Society of Environmental Bioindicators (ISEBI) has emphasized the necessity to develop bioindicators and biomarkers as "sentinels" to discover and locate the presence of any disturbances that may have an impairment effect on the different components of the environment and enable elucidation of their spatial and the temporal effects.

Y. Steinberger (✉)
The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University,
Ramat-Gan, 52900, Israel
e-mail: steinby@mail.biu.ac.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_8,

These scientific paradigms may be any biological organization that can detect biomarkers which respond to anthropogenic compounds. The biological systems may vary from a cellular to community to ecosystem level, from low to high ecological relevance and from short term to long term response (McCarty and Munkittrick 1996).

The present chapter describes such a scientific paradigm, that of soil biota, which has universal application. The soil biota, i.e., the microflora, microfauna, and mesofauna, have the ability to reflect the changes in soil since they live in close contact with the different soil components as well with the belowground parts of plants (e.g., roots). There are many possible ways to evaluate the bioavailability of "pollutants" in soil. The most widely used methods can be direct, i.e., measure the concentration inside the organisms collected from the soil or indirect, e.g., monitor changes in population size, biomass, CO_2 evolution, total diversity, functional diversity, etc. In addition, to the microflora, microfauna, and mesofauna, abiotic factors, i.e., the type of soil and its permeability, play a role in the transport of anthropogenic compounds in the soil.

8.1.1 Abiotic Components

The abiotic components of a terrestrial ecosystem are the non-living features of the ecosystem that the living organisms depend on. The topsoil (~0–20 cm) is known as the soil layer affected by physical, biological and sometimes chemical degradation. Such change affects both soil biota and plant communities. The abiotic soil components include mineral matter, water, air and organic matter which are disposed to factors such as temperature, light, rainfall, atmospheric gases and wind. Air and water percentages vary significantly with soil texture, weather and plant water uptake. The mineral component is composed of various proportions of sand, silt and clay particles. Sand particles are 0.05–2 mm in diameter, silt particles are 0.002–0.05 mm in diameter and clay particles are less than 0.002 mm in diameter. Because clay particles have a very large surface area to volume ratio, they can hold much more water and nutrients than larger particles. Water and air in the soil is mainly determined by the different physical and chemical properties, where the soil texture is an important determinant of water retention, bulk density, aeration and fertility. Moreover the water air ratio plays an important role in soil population activity and trophic relation. Soil organic matter is one of the most important components of ecosystems, although soil organic matter usually comprises less than 5% of a soil's weight. Spatial and temporal changes in soil organic matter modify soil biota habitats and trophic composition depending on its quality and quantity. Organic matter inputs from a wide range of sources, both aboveground and belowground parts (annual plants, perennial plants, roots) which have significant C/N, lignin, chemical composition and maturity will determine its decomposition-breakdown rates by a complex interaction between the soil biota components. As described below when biota feed on the soil organic matter, they also contribute inorganic nutrients such as, carbon, nitrogen, sulfur, phosphorus and trace elements to the soil. Below the topsoil is the unsaturated zone which reaches from topsoil to the groundwater which is termed the vadose zone. Although poor in biological activity, the mass of soil in the vadose zone and its physical chemical characteristics may be a major factor in whether a chemical reaches the groundwater.

8.1.2 Biotic Components

The biotic components of any ecosystem contain all the living organisms and the mutual influences and trophic relations between them. The three main compartments of a food chain are: (1) producers – plants – which have the ability to assemble organic compounds from inorganic material, (2) consumers – the organisms that use organic matter without being able to transform organic mater from one form to another (Fig. 8.1), and (3) decomposers-organisms (microflora, e.g., bacteria and fungi) that obtain energy by converting the organic matter generated from the producers and consumers into inorganic nutrients which can be reused by the producers. The consumers are divided according to their food consumption (a) herbivores – plant feeders; (b) carnivores or predators feeding on meat and (c) omnivores – being able to feed both on plants and animal. Two main processes that connect all the three main parts of the ecosystem are: (a) energy flow and (b) nutrient flow (Fig. 8.1).

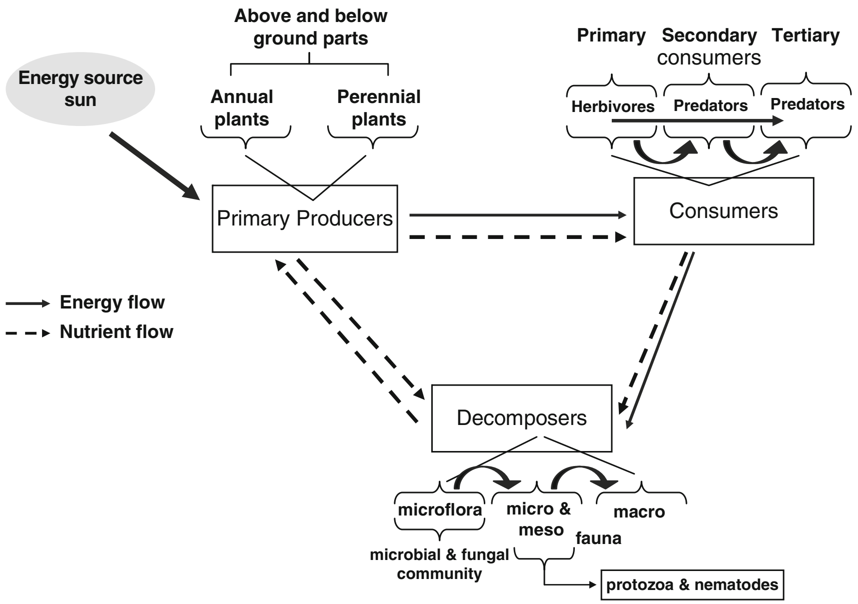


Fig. 8.1 The biotic components of the soil ecosystem the mutual influences and trophic relations between organisms

The energy "flow" is the driving current that runs the system. The absolute energy source is the "sun," which triggers the whole flow that determines life functions such as movement, growth and reproduction. Only at the first step at "sun-plant" assembly is the sun energy converted to chemical energy to become a structural part of the plant. The energy incorporated into the plant is then a potential food source for the two food chain compartments – the consumers and the decomposers.

The accumulation of organic matter from the different compartments of the food chain (detritus) is used by the decomposers where energy produced by breakdown is released and organic compounds are mineralized into inorganic components. The inorganic components are reused by plants – forming a "closed circle recycling" nutrient flow.

The carbon and nitrogen cycle are the most informative in determining the mutual interdependence between producers, consumers and decomposers due to their great importance in assembling protein and other nitrogenous compounds, e.g., nucleic acids. The carbon cycle is defined as all the organic compounds containing carbon and the most important source of all inorganic carbon, atmospheric CO_2. Atmospheric CO_2 is used by the autotrophic organisms, primarily plants via photosynthesis, to assemble carbohydrates, protein, fats, etc. The nitrogen cycle also relies on the atmosphere (which is 78% nitrogen) for the basic element essential for nucleic acids, proteins and other nitrogenous compounds. However, unlike CO_2, only a few groups of bacteria can use nitrogen in its gaseous form and transform it into a useable form. Therefore most of the nitrogen available for use is in the form of nitrates from plants or ammonia resulting from breakdown of organic matter (proteins) by decomposers via the ammonification process. The ammonia is then converted to nitrite by bacteria (*Nitrosomonas* and *Nitrosococcus*) and subsequently from nitrites to nitrates by bacteria (*Nitrobacter*) – a process known as nitrification.

8.2 Measuring Soil Biotic Activity and Biodiversity as Bioindicators

Soil is a complex system in which the physical and chemical components play an important role in soil quality determination. A high level of biodiversity and activity is recognized as a good indicator of soil health. The activity of soil biota is crucial for soil function, although the interactions between the organisms are very complex (Coleman and Crosley 1996). The role of particular organisms can be deduced in a variety of ways, which can allow the individual organisms to be arranged into functional groups. Conversely, any changes in soil physical and chemical composition may be used to eliminate a specific group or groups of organisms. Changes in population size, activity, biomass, diversity, trophic group relationship can be followed in soil and correlated with particular processes (Wood 1995; Mulder 2006).

Doran et al. (1994) have proposed the suitable parameters of a soil biological indicator organism. The organism should (1) reflect some aspect of the functioning of the ecosystem, (2) show a prompt and accurate response to perturbation, (3) should be potentially of universal and natural abundance, and (4) easy to collect and handle. There should be a detailed understanding of the trophic significance and of how the individual organism will respond to any temporal and spatial changes of the environment. The combination of these parameters could then allow implementations of long-term strategies in soil management

One of the best documented soil bioindicators that fit the above parameters is the soil microbial community (Fig. 8.2). As defined by Lynch et al. (2004), the soil microbial community is a complex mixture that may contain as many as 10,000 different species in a single gram of soil. In order to be able to evaluate changes in the microbial community as result of perturbation, the following quantitative measurements are of great consequence: biomass, CO_2 evolution, N transformation, biomass C, diversity, functional diversity, a variable that allows quantification of the environmental impact (qCO2) and the contribution of microbial biomass to soil organic carbon (Cmic/Corg) (Anderson and Domsch 1989; Anderson 2003).

A second important group is the micro and mesofauna because of their fundamental importance in nutrient cycling, their abundance and diversity, in particular, the nematodes. Numerous studies have established the potential of soil free living nematodes as indicators because they inhabit virtually all ecosystems including marine, freshwater, and terrestrial environments. The nematodes occupy a central position in the soil food web occurring at multiple trophic levels (bacteria feeders, fungi feeders, plant feeders and parasites, predators and omnivor) and, therefore, have the potential to provide insights into the condition of soil food webs (Neher

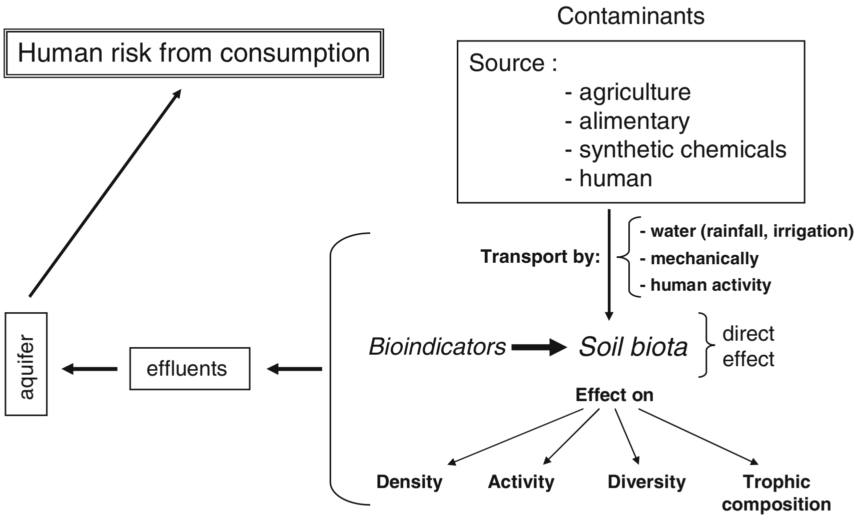


Fig. 8.2 The role of the soil microbial community as bioindicators

2001). The nematode fauna displays distinct patterns in abundance and composition that are related to soil type, but also are strongly influenced by agricultural soil use. Therefore data on the abundance and diversity of soil nematodes allows long term comparisons for a variety of changes in soil composition.

With the dataset available, the main question however remains whether we can assess the health of the soil biodiversity. Strictly speaking, no guidance comes from just determining values for parameters at a given location. These values should be benchmarked against a certain reference value, in order to assess soil biological health (e.g. judge it to be bad, normal or healthy) and to guide policy measures.

The immense diversity of microorganisms in even the simplest soils presents major technological problems. Identification of specific DNA sequences (Borneman et al. 1996) or ribosomal 16s DNA (Sanguin et al. 2006) have both yielded useful information. Development of suitable microarrays will mean a substantial advance as the technology eliminates the use of PCR and it can be used by a large number of laboratories and tens of thousands of oligonucleotides can be placed on a chip (Small et al. 2001). Substrate utilization testing relies on the observation that various bacterial groups use specific substrates. Substrate utilization tests can also assay the activity of specific enzymes like dehydrogenase, urease and xylinase (Kandeler et al. 1999; Carpenter-Boggs et al. 2000). Substrate induced respiration (SIR) has the advantage that, unlike the molecular methods, only live bacteria present are measured. The SIR divided by the basal respiration rate is designated as the qCO_2.

Products of microorganisms can also be used to profile the soil community. Ergosterol is produced by fungi and is used as a parameter of fungal mass (Montgomery et al. 2000). Beta sitosterol is a widespread plant compound while cholesterol is typical of eukaryotes. Phospholipid ester-linked fatty acids (PLFAs), which make up the cell membrane, and respiratory quinones are produced by all microbes and are thought to be rapidly destroyed, thus they reflect “live” bacteria (Chun et al. 2006). The coprostanols (Chap. 7) would indicate the presence of fecal compounds.

8.3 Measuring the Impact of Manure and Effluents from CAFOs on Soil Biodiversity

Most of the literature dealing with the effects of organic farming on soil biodiversity has focused on the use of composts, sludges and human domestic waste. Composting destroys most organic compounds such as the steroids and pharmaceuticals generated from CAFOs so the effects of these compounds would be negligible compared to untreated manure or effluents (Hakk et al. 2005; Büyüksönmez and Sekeroglu 2005). Manures are generally applied as a slurry from the sty or barn. Some manures, in particular chicken manure, are collected as solids and distributed using a manure spreader.

In general, farmyard manure increases microbial biomass as measured by respiration, N-mineralization, urease, arginine deaminase, alkaline phosphatase activity compared to inorganic fertilization (Kandeler et al. 1999). Slurries from CAFOs contain large amounts of ammonia. It would therefore be expected that the ammonia oxidizing bacteria would be most affected by the slurry.

8.3.1 Swine Manure and Slurry

In a study of pig slurry, Hastings et al. (1997) using 16S rDNA-directed PCR reported that members of the genus *Nitrosomonas* were detected only in those soil plots that had received high loadings of slurry while *Nitrosospira* were not affected. The presence of the ammonia monooxygenase gene (*amoA*) of *Nitrosomonas europaea* increased with the amount of pig slurry applied to plots, while it was undetected in an untreated plot. Plaza et al. (2004) found that amending soil with pig slurry over a four year period over the range of 30, 60, 90, 120 and 150 m/ha/year resulted in a linear increase in dehydrogenase, catalase and β-glucosidase and a decrease in phosphatase activity. On the other hand, there was little change in basal respiration and qCO_2 decreased.

8.3.2 Cattle Manure and Slurry

Dry cattle manure applied over several years resulted in increased microbial respiration and SIR (Enwall et al. 2007). However, due to the low pH of the soil amended with manure, the qCO_2 decreased. There was an increase in ammonia oxidizing activity and there was a unique profile of 16s rRNA genes, which represent the bacterial community, compared to other treatments. Using composted cattle manure in laboratory experiments, Saviozzi et al. (2006) reported the material increased dehydrogenase activity, C mineralization and total microbial activity.

We have found that under anaerobic conditions under a cattle waste lagoon described in Chap. 6, that microbial biomass was significantly less in the hormone loaded soils than a reference site in both the upper clay and unsaturated lower sandy layers of the vadose zone. The saturated clay layer had a shift in bacteria utilizing carboxylic and protein substrates and reduction in aromatics compared to the reference site (Alon, Steinberger, Shore, preliminary observations). In contrast, under aerobic conditions, addition of progesterone, estradiol or testosterone to soils that had been amended with cattle manure resulted in an increase in microbial respiration while the same steroids had no affect on unamended soil (Alon, Steinberger, Shore, preliminary observations).

8.4 Summary

Many parameters of soil ecology like organic matter and species diversity can be measured. The soil microbial community, being most vulnerable to hormones and pharmaceuticals, could be a valuable tool to explaining how these compounds move through and affect the soil. However, the literature of the effects of effluents from CAFOs is sparse, e.g., nothing is known on how fertilization with chicken manure, so widely used in Arkansas and Maryland, affects the soil microbial community. What is known about effluents containing specific pharmaceuticals and hormones on the soil ecology is discussed in the subsequent chapters.

References

Anderson TH (2003) Microbial eco-physiological indicators to asses soil quality. Agric Ecosyst Environ 93:285–293

Anderson TH, Domsch KH (1989) Ratio of microbial biomass carbon to total organic carbon in arable soils. Soil Biol Biochem 4:471–479

Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JL, Nienhuis J, Triplett EW (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. Appl Environ Microbiol 62:1935–1943

Büyüksönmez F. Sekeroglu S (2005) Presence of pharmaceuticals and personal care products in biosolids and their destruction during composting. J Residuals Sci Technol 2:31–40

Carpenter-Boggs L, Kennedy AC, Reganold JP (2000) Organic and biodynamic management: effects on soil biology. Soil Sci Soc Am J 64:1651–1659

Chun S, Lee J, Radosevich M, White DC, Geyer R (2006) Influence of agricultural antibiotics and 17β-estradiol on the microbial community of soil. J Environ Sci Health B 41:923–935

Coleman DC, Crosley Jr DA (1996) Fundamentals of soil ecology. Academic Press, New York, 205 pp

Doran JW, Coleman DC, Bezdicek DF, Stewart BA (1994) Defining soil quality for a sustainable environment. Soil Science Society of America Special Publication Number 35, ASA, Madison, WI

Enwall K, Nyberg K, Bertilsson S, Cederlund H, Stenström J, Hallin S (2007) Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biol Biochem 39:106–115

Hakk H, Millner P, Larsen G (2005) Decrease in water-soluble 17β-estradiol and testosterone in composted poultry manure with time. J Environ Qual 34:943–950

Hastings RC, Ceccherini MT, Miclaus N, Saunders JR, Bazzicalupo M, McCarthy AJ (1997) Direct molecular biological analysis of ammonia oxidising bacteria populations in cultivated soil plots treated with swine manure. FEMS Microbiol Ecol 23:45–54

Kandeler E, Stemmer M, Klimanek E-M (1999) Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biol Biochem 31:261–273

Lynch JM, Benedetti A, Insam H, Nuti MP, Smalla K, Torsvik V, Nannipieri P (2004) Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol Fertil Soils 40:363–385

McCarty LS, Munkittrick KR (1996) Environmental biomarkers in aquatic toxicology: fiction, fantasy or functional. Human Ecol Risk Assess 2:268–274

Montgomery HJ, Monreal CM, Young JC, Seifert KA (2000) Determination of soil fungal biomass from soil ergosterol analyses. Soil Biol Biochem 32:1207–1217

Mulder C (2006) Driving forces from soil invertebrates to ecosystem functioning: the allometric perspective. Naturwissenschaften 93:467–479

Neher DA (2001) Role of nematodes in soil health and their use as indicators. J Nematol 33:161–168

Plaza C, Hernández D, Garcıá-Gil JC, Polo A (2004) Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biol Biochem 36:1577–1585

Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moënne-Loccoz Y, Grundmann GL (2006) Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial communities. Appl Environ Microbiol 72:4302–4312

Saviozzi A, Cardelli R, N'kou P, Levi-Minzi R, Riffaldi R (2006) Soil biological activity as influenced by green waste compost and cattle manure. Compost Sci Util 14:54–59

Small J, Call DR, Brockman FJ, Straub TM, Chandler DP 2001 Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. Appl Environ Microbiol 67:4708–4716

Wood M (1995) Environmental soil biology. Chapman & Hall, The University Press, Cambridge, 150 pp

Chapter 9
Occurrence and Transport of Antibiotics from CAFOs

Amy Pruden

Abstract The largest source of antibiotics in the environment is likely their application in livestock. In particular, their routine use as growth promoters comprises the majority of antibiotic use. Antibiotic use for growth promotion is widespread in the US, but banned in Israel, Denmark, and other parts of Europe. Antibiotics consumed by livestock are largely unmetabolized and excreted into the environment. This chapter will discuss the various sources of antibiotics and their transport in the environment with respect to their chemical characteristics. Potential impacts of antibiotics in the environment will also be discussed.

9.1 Introduction

In the US it is estimated that about half of the antibiotics used in the country are administered to livestock (Mellon et al. 2001). These are used for both therapeutic and subtherapeutic purposes. Therapeutic use refers specifically to the application of antibiotics to treat observed illness. Subtherapeutic use is defined by application when the animal is not necessarily ill and can have one of two main purposes. The first is prophylactic use intended to prevent expected onset of disease or to protect livestock from a known outbreak. The second purpose is to promote weight gain by optimizing the feed conversion ratio. Subtherapeutic antibiotic use for both purposes plays an especially key role in CAFOs where animals are kept in close proximity and thus are more susceptible to illness and where maximizing production efficiency is the key goal. Because of concern of the potential role of antibiotic overuse in livestock and the rise of antibiotic resistance, subtherapeutic use of antibiotics was banned in the EU in 2006. The topic of antibiotic resistance will be

A. Pruden (✉)
Via Department of Civil & Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA, 24061, USA
e-mail: apruden@vt.edu

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_9,

addressed in detail in Chap. 10. In this chapter we will examine antibiotic use, occurrence in the environment and overall fate and transport.

9.2 Antibiotic Use in CAFOs

Antibiotics have been administered to livestock in the US since the 1940s when it was discovered that tetracycline fermentation by-products led to increased weight gain in chickens (Stokstad and Jukes 1958–1959). Since that time, antibiotic use in livestock has boomed. It has been estimated that in the US, 36 million pounds of antibiotics are administered to livestock annually, and that only 30% of this use is to treat specific diseases (Mellon et al. 2001). The Animal Health Institute has put forward a more conservative estimate, indicating that 18 million pounds of antibiotics are used and 83% of these are to treat specific diseases (Viola and DeVincent 2006). In any case, it is difficult to obtain accurate estimates because pharmaceutical companies are not required to report quantities of veterinary drugs that are manufactured and sold. Also, many of the antibiotics may be purchased over the internet, without any regulatory oversight. Table 9.1 provides a cross section of several of the antibiotics approved for livestock in the US. A complete list of approved drugs is published online by the USFDA (http://www.fda.gov/cvm/greenbook.html).

There is no doubt that antibiotic use in livestock has had tremendous benefits, especially in the CAFO environment. In most direct terms, there is a clear relationship between antibiotic use and a higher feed conversion ratio. The feed conversion ratio is defined as the weight gained per pound of feed consumed. Higher efficiency has the net effect of bringing animals to market quicker. For example, in the 1950s 84 days were required to produce a 5 lb chicken while today only 45 days are required (HSUS 2006). In the case of beef cattle on US feedlots, it is estimated that they are brought to market 60 days sooner because of antibiotic use.

The higher feed conversion ratio associated with antibiotic use is a result in part of disease prevention, but is most directly a result of increased absorption of feed. This is accomplished by inhibiting cocci bacteria in the gut microflora. Ionophores such as monensin shift the gut microflora to favor propionate rather than acetate production and to decrease methane formation. This results in an overall increase in the metabolic energy value of the feed (Dowling 2006). Ionophore anticoccidials have proven particularly effective for promoting weight gain in poultry and beef. Recently monensin, under the trademark Rumensin®, was also approved for use in dairy cattle in the US (US FDA 2004). Because of their effectiveness, specially formulated feeds that incorporate growth promoting antimicrobials, such as Rumensin®, have become commonplace. Interestingly, the concentrations of ionophores typically administered to poultry are actually toxic to cattle, sheep and rabbits (EFSA 2008). Maduramicin is a toxic compound and can be present in poultry litter in concentrations that are lethal to cattle fed the silaged poultry litter (Shlosberg et al. 1997).

Table 9.1 Summary of antimicrobials approved for livestock in the US

Class of antimicrobials	Representative antimicrobials	Swine	Poultry	Dairy cattle	Beef cattle
B-lactam	Amoxicillin	✓		✓	✓
	Ampicillin	✓			
	Penicillin	✓	✓	✓	
	Ceftiofur	✓	✓	✓	✓
Macrolides	Erythromycin	✓	✓	✓	✓
	Tylosin	✓	✓	✓	✓
	Oleandomycin	✓			
Tetracylines	Tetracycline	✓	✓	✓	
	Chlortetracycline	✓	✓	✓	✓
	Oxytetracyline	✓	✓	✓	✓
Aminoglycosides	Apramycin	✓			
	Dihydrostreptomycin	✓		✓	✓
	Gentamicin	✓			
	Neomycin	✓	✓	✓	
	Spectinomycin	✓	✓		
	Streptomycin	✓	✓		
Peptides	Bacitracin	✓	✓	✓	
Licosamides	Lincomycin	✓	✓		
Fluoroquinolones	Enrofloxacin				✓
Ionophores	Monensin		✓	✓	✓
	Narasin		✓		
	Salinomycin		✓		
Sulfonamides	Sulfachloropyridizine	✓		✓	
	Sulfadimethoxine		✓	✓	✓
	Sulfamethazine	✓	✓	✓	✓
	Sulfaquinoxaline		✓	✓	✓
	Sulfathiazole	✓			
Misc	Ormethoprim		✓		
	Carbodox	✓			
	Etrotomycin	✓			
	Bambermycins	✓	✓	✓	
	Tiamulin	✓			
	Tilmicosin	✓		✓	✓
	Virginamycin	✓	✓		

Compiled from the FDA Database of Approved Animal Drug Products: http://dil.vetmed.vt.edu/NADA/

9.3 Occurrence, Fate, and Transport

9.3.1 Occurrence and Fate

Despite their benefits, there is growing concern about environmental, human, and animal health impacts of agricultural antibiotics. Antibiotics in general are poorly broken down once ingested and significant portions are excreted in manure unaltered and as metabolites (Elmund et al. 1971; Boxall et al. 2003, 2004). Several studies have confirmed the presence of antibiotics in animal manure, lagoons, compost, soil, and surface water. In 2004, antibiotics of several classes were analyzed in seven dairy lagoons and manure stockpiles (Carlson et al. 2004). The lagoon samples ranged from non-detectable (ND) levels to 17 parts per billion tetracyclines, ND to 17 parts per billion sulfonamides, and 19 parts per billion for macrolides. The solid manure samples also ranged from non-detectable levels to 5,130 parts per billion tetracyclines, ND to 46 parts per billion of sulfonamides, and ND to 5 parts per billion macrolides. A recent survey of treated drinking water across the US indicated that pharmaceuticals, including antibiotics, were present in over half of the water supplies (AP 2008). In a study of sulfonamides conducted in Berlin, Germany, the concentrations ranged from <0.02–50.8 μg/L in wastewater, <0.02–41 μg/L in groundwater below a former sewage farm, <0.02–1.15 μg/L in surface water, and <0.02–0.27 μg/L in drinking water (Richter et al. 2007). Realization of the presence of antibiotics in drinking water has been a significant wake-up call of the pervasiveness of these compounds, though the exact impacts, if any, are not clear.

Figure 9.1 provides an overview of the major sources and pathways of antibiotics in the environment, including human sources. As manure is stored, antibiotics can degrade further. Tylosin in pig slurry and penicillin in poultry manure have been observed to persist for days, while chlortetracycline can persist for months

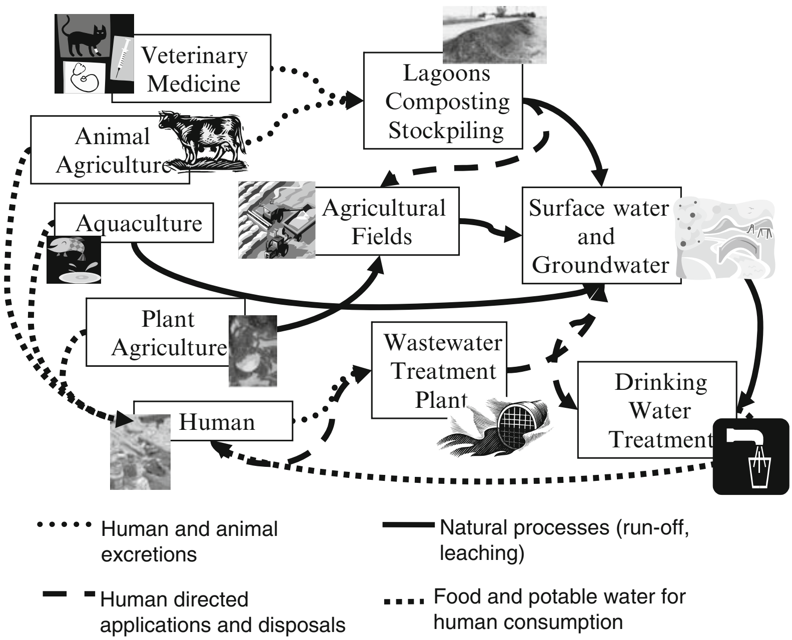


Fig. 9.1 Overview of origins and pathways of antibiotics in the environment (artistic credit to Heather Storteboom)

(Gavalchin and Katz 1994; Loke et al. 2000). In a study of stockpiled beef manure, half-lives of chlortetracycline, tetracycline, and oxytetracycline were estimated to be 13.5, 17.2, and 31.1 days, respectively (Storteboom et al. 2007). Persistence can also vary across manure types. In the same study by Storteboom and colleagues, the half-life of chlortetracycline was 6.8 days in stockpiled dairy manure and 8.4 days in horse manure where chlortetracycline was artificially spiked. While Storteboom et al. (2007) found a slight advantage of composting over stockpiling for antibiotic degradation, a recent study by Dolliver et al. (2008) did not find any significant difference in the degradation rates of chlortetracycline, monensin, sulfamethazine, or tylosin in spiked turkey litter that was either stockpiled and composted by two different methods. Assuming first-order decay, the half-lives for chlortetracycline, monensin, and tylosin were 1, 17, and 19 days, respectively. Thus, chlortetracycline degraded much faster in this study than the Storteboom study. There was no evidence of sulfamethazine degradation. Sulfachloropyridazine has been observed to rapidly degrade in the feces of broiler hens but persist in laying hens (Van Dijk and Keukens, 2000). It is also important to consider that metabolites may revert to the active parent compound in the manure (Boxall et al. 2003).

Depending on local practices and regulations, animal manure is often subject to solids separation, held in lagoons, and the residuals of both subsequently composted and/or land applied. Each step of this process has the potential to contaminate soil and surface water with residual antibiotics present in the manure. While the goal is typically to retain all animal waste within the boundaries of the livestock operation, unintentional discharges of overland flow to surface water can take place, especially during storm events. In the US, discharge permits are required of many large-scale operations as a precaution in case of unintentional discharge. Leaching of antibiotics to groundwater from lagoons and land-applied residuals has also been observed. For example, concentrations of sulfamethazine were found from 0.076 to 0.22 μg/L and sulfadimethoxine from 0.046 to 0.068 μg/L in well water neighboring a beef CAFO (Batt et al. 2006). Lindsey and colleagues detected sulfamethoxazole in groundwater at 0.22 μg/L, but did not find tetracyclines among the 6 groundwater and spring water samples tested (Lindsey et al. 2001). They hypothesized that the relative hydrophobicity of tetracyclines limits their entry to groundwater.

As would be expected, antibiotics are detected in surface water much more frequently than in groundwater. Lindsey and colleagues found chlortetracycline at 0.15 μg/L, oxytetracycline ranging from 0.07–1.34 μg/L, and tetracycline at 0.11 μg/L among 138 surface water samples tested. In the landmark survey of pharmaceuticals in streams conducted by Kolpin et al. (2002), the maximum concentration detected of chlortetracycline was 0.68 μg/L, oxytetracycline was 0.34 μg/L, and tetracycline was 0.11 μg/L. Because some of the same antibiotics are used in both human and veterinary medicine, it can be difficult to determine the original source of an antibiotic once found in a surface water body. However, some antimicrobials, particularly ionophores, are used exclusively in agriculture and thus provide highly suitable markers for livestock sources. In a study by Kim and Carlson (2006) of the mixed landscape Cache La Poudre River in northern Colorado monensin, salinomycin, and narasin were present at concentrations up to 0.04 μg/L in water and 30 μg/kg in sediments. Tylosin, which is also solely used for agricultural purposes, was detected in 13.5% of streams tested by Kolpin et al. (2002) at a maximum concentration of 0.28 μg/L.

9.3.2 Transport

The actual mechanisms of transport vary between antibiotics depending on their chemical characteristics. For example, sulfonamides are generally among the most water soluble among the antibiotics commonly used in agriculture, thus it is not surprising that they are relatively easily detectable in groundwater (Batt et al. 2006) and surface water (Kolpin et al. 2002). Davis et al. (2006) recently performed a comprehensive study of the transport of seven different antibiotics (tetracycline, chlortetracycline, sulfathiazole, sulfamethazine, erythromycin, tylosin, and monensin) during a simulated rainfall event. The study design allowed for determination of losses associated with overland flow versus sediment transport. Pseudo-partitioning coefficients were determined for all antibiotics as an indicator of their association with the sediment versus the aqueous phase. As expected, losses of the two sulfonamide antibiotics, which were the first and third most water soluble of the antibiotics tested, were primarily associated with the aqueous phase. Estimated losses of only 5% and 23% of the total loss were associated the sediment for sulfamethazine and sulfathiazole, respectively. Interestingly, though monensin was the most hydrophobic of the antibiotics investigated, its losses were also primarily associated with the aqueous phase, with only 9% of total loss associated with the sediments. However, monensin also had the greatest total losses of any antibiotics, at about 0.08%. Relative losses for the other antibiotics ranged from 0.002% for tetracycline to 0.049% for erythromycin. The losses of the second and third most hydrophobic antibiotics tested, erythromycin and tylosin, were primarily associated with the sediment as expected (74 and 77%, respectively). The overall conclusion was that erosion control can help to retain some antibiotics, but not all. Also, antibiotics of the same class do not necessarily exhibit identical behavior. Octanol–water partitioning coefficients provide some guidance in predicting fate and transport, but do not tell the whole story.

The above study by Davis and colleagues represented a “worst case scenario” for antibiotic transport because the antibiotics were sprayed onto the plots followed by a relatively intense simulated rain event. A more recent study by Dolliver and Gupta (2008a) monitored the fate of antibiotics over a 3 year period from land-applied hog and beef manures comparing chisel plowing and no-tillage systems. The hog manure was in liquid form and contained chlortetracycline and tylosin while the beef manure was in solid form and contained chlortetracycline, tylosin and monensin. Relative mass losses of the three antibiotics was found to be <5%. Monensin and tylosin were present in both leachate and runoff, but chlortetracycline was only detected in runoff. As was observed in the study by Davis, monensin concentrations were particularly high, up to 40.9 μg/L in leachate and 57.5 μg/L in runoff. The highest concentrations measured of tylosin was 1.2 μg/L in leachate and 6.0 μg/L in runoff. The highest concentration of chlortetracycline detected was 0.5 μg/L. It was found that most of the antibiotic losses occurred during the non-growing season, which is logical considering that this is immediately following fall application of the manure and biological degradation is hampered due to reduced temperatures. In this study, no-tillage actually resulted in increased losses due to deeper percolation. One of the years studied was noted to have a particularly high level of snow melt, and 100% of antibiotic losses that year were due to runoff. In the other 2 years, only about 40% of the total loss was due to runoff. In a parallel study by the same authors (Dolliver and Gupta 2008b) it was found that runoff from manure stockpiles can result in extremely high concentrations of antibiotics. In this study of beef manure, the highest concentrations detected of chlortetracycline, monensin, and tylosin 210, 3,175, and 2,544 μg/L, respectively.

9.4 Summary

Thus, overall it can be concluded that though some on-farm antibiotic degradation can occur, agricultural antibiotics do make their way to groundwater and surface water, though surface water is more directly affected. Also, the chemical properties of the antibiotic, in particular its relative hydrophobicity, plays a significant role in governing its fate. Thus, the relatively water soluble sulfonamides are more commonly detected in groundwater and surface water. However, relatively hydrophobic monensin also is detected frequently and at relatively high concentrations in both runoff and leachate. Also, transport behavior is not uniform within a given class of antibiotics. Therefore, while general patterns of fate and transport of antibiotics in the environment have been confirmed, there are still mechanistic details that are not fully understood and require further study.

References

AP (Associated Press) (2008) AP investigation-water supplies contaminated with drugs. Published March 10, 2008. Available in Archives at: http://www.aparchive.com

Batt AL, Snow DD, Aga DS (2006) Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. Chemosphere 64:1963–1971

Boxall ABA, Kolpin DW, Halling-Sorensen B, Tolls J (2003) Are veterinary medicines causing environmental risks? Environ Sci Technol 37:286A–294A

Boxall ABA, Fogg LA, Blackwell PA, Kay P, Pemberton EJ (2004) Veterinary medicines in the environment. Rev Environ Contam Toxicol 180:1–91

Carlson KS, Yang S, Cha J-m, Doesken K, Davis J (2004) Antibiotics in animal waste lagoons and manure stockpiles. From the Ground Up Agronomy News 24(3):7–8. Colorado State University Cooperative Extension, Fort Collins, CO. Available online at http://www.extsoil-crop.colostate.edu/Newsletters/2004/Antibiotics/index.html (verified 23rd May 2008)

Davis JG, Truman CC, Kim SC, Ascough JC, Carlson K (2006) Antibiotic transport via runoff and soil loss. J Environ Qual 35:2250–2260
Dolliver H, Gupta S, Noll S (2008) Antibiotic degradation during manure composting. J Environ Qual 37:1245–1253
Dolliver H, Gupta S (2008a) Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J Environ Qual 37:1227–1237
Dolliver H, Gupta S (2008b) Antibiotic losses from unprotected manure stockpiles. J Environ Qual 37:1238–1244
Dowling PM (2006) Miscellaneous antimicrobials: ionophores, nitrofurans, nitromidazoles, firamycins, oxazolidinones, and others. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM (eds) Antimicrobial therapy in veterinary medicine, 4th edn. Blackwell, Ames, IOWA
Elmund GK, Morrison SM, Grant DW, Nevins MP (1971) Role of excreted chlortetracycline in modifying the decomposition process in feedlot waste. Bull Environ Contam Toxicol 6:129–132
EFSA (European Food Safety Authority) (2008) Cross-contamination of non-target feedingstuffs by maduramicin authorised for use as a feed additive. The EFSA Journal 594:1–30
Gavalchin J, Katz SE (1994) The persistence of fecal-borne antibiotics in soil. J AOAC Int 77:481–485
HSUS (2006) The welfare of animals in the broiler chicken industry. In: An HSUS Report: HSUS (ed). HSUS: Washington, DC, pp 1–7 Available online at http://www.hsus.org/farm/resources/research/welfare/broiler_industry.html (verified 23 May 2008)
Kim SC, Carlson K (2006) Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. Water Res 40:2549–2560
Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211
Loke ML, Ingerslev F, Halling-Sørensen B, Tjornelund J (2000) Stability of Tylosin A in manure containing test systems determined by high performance liquid chromatography. Chemosphere 40:759–765
Lindsey ME, Meyer M, Thurman EM (2001) Analysis of trace levels of sulfonamide and tetracycline antimicrobials, in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Anal Chem 73:4640–4646
Mellon MG, Benbrook C, Benbrook KL (2001) Hogging it: estimates of antimicrobial abuse in livestock. Union of Concerned Scientists, Cambridge, MA
Richter D, Dunnbier U, Massmann G, Pekdeger A (2007) Quantitative determination of three sulfonamides in environmental water samples using liquid chromatography coupled to electrospray tandem mass spectrometry. J Chrom A 1157:115–121
Shlosberg A, Perl S, Harmelin A, Hanji V, Bellaiche M, Bogin E, Cohen R, Markusfeld-Nir O, Shpigel N, Eisenberg Z, Furman M, Brosh A, Holzer Z, Aharoni Y (1997) Acute maduramicin toxicity in calves. Vet Rec 140:643–646
Stokstad ELR, Jukes TH (1958–1959) Studies of the growth-promoting effect of antibiotics in chicks on a purified diet. Antibiot Annu 6:998–1002
Storteboom HN, Kim SC, Doesken K, Davis J, Carlson KH, Pruden A (2007) Response of antibiotics and resistance genes to high-intensity and low-intensity manure management. J Environ Qual 36:1695–1703
US FDA (US Food and Drug Administration) (2004) FDA approves Rumensin® for increased milk production efficiency in dairy cows. Report available online at http://www.fda.gov/cvm/CVM_Updates/monensinup.htm (verified 23 May 2008)
Van Dijk J, Keukens HJ (2000) In: Residues of veterinary drugs in food: proceedings of the Euroresidue IV conference, Van Ginkel LA, Ruiter A (eds), Veldhoven, The Netherlands
Viola C, DeVincent SJ (2006) Overview of issues pertaining to the manufacture, distribution, and use of antimicrobials in animals and other information relevant to animal antimicrobial use data collection in the United States. Prev Vet Med 73:111–131

Chapter 10
Antibiotic Resistance Associated with CAFOs

Amy Pruden

Abstract The major concern of widespread antibiotic use in agriculture is not necessarily the compounds themselves, but their potential to contribute to the emergence and spread of antibiotic resistance. The rate of occurrence of multi-drug resistant pathogens, such as tuberculosis and MRSA, continues to rise at a rapid rate, leading to growing concern in the health care realm. A growing body of multi-disciplinary research, in particular in microbial ecology and environmental science, is building an understanding of the relationship between antibiotics and antibiotic resistance in the environment. One particular view is that the antibiotic resistance genes themselves are emerging contaminants for which strategies are needed to treat and contain them. At the same time, it is important to keep in mind that antibiotic resistance genes, like many antibiotics, are naturally occurring. Therefore distinguishing anthropogenic influence from the natural background is essential. This chapter will tie together research from various disciplines to shed light on how antibiotic resistance is generated and transported in the environment.

10.1 Introduction

Recently public concern has been renewed regarding CAFOs and antibiotic resistance. The Pew Charitable Trusts partnered with John's Hopkins University recently released a comprehensive report in 2008 compiled with the assistance of a cross-section of stakeholders on the topic of industrial farm animal production (IFAP) in the US. In this report it was recommended that subtherapeutic use of antibiotics in animal agriculture (see Chap. 9 for definitions) be phased out in the US, as has recently occurred in the EU. This recommendation is primarily driven by increasing evidence of the association of antibiotic resistance with CAFOs. Other entities that have recommended a ban

A. Pruden (✉)
Via Department of Civil & Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA, 24061, USA
e-mail: apruden@vt.edu

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_10,

on subtherapeutic antibiotic use in the US include the American Medical Association, the American Society for Microbiology, and the American Public Health Association. In this chapter, we provide an overview of the rising problem of antibiotic resistance, explain the various mechanisms of resistance, and describe the potential role of CAFOs in exacerbating the antibiotic resistance problem.

10.2 The Problem of Antibiotic Resistance

Antibiotics have revolutionized human health care, but in recent years their effectiveness has been diminishing. We know today that the rate of antibiotic resistance among many disease-causing bacteria has been increasing every year. This means that antibiotics are not as reliable as they once were in fighting disease. In the US it is estimated that about 98,000 patients die each year from hospital acquired infections (Klevens et al. 2007), which is a notable increase from 13,300 deaths in 1992 (Cardo et al. 2004). According to most recent estimates, 70% of all hospital acquired infections are resistant to at least one class of antibiotic (Leeb 2004).

Some antibiotic resistant bacteria (ARB) that infect humans have been gaining considerable public attention. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) was once thought to only occur in hospitals, but is now also being traced to the general community (community-associated resistance) (CDC 2006). From 1999–2005, the estimated number of MRSA related hospitalizations more than doubled, from 127,036 to 278,203 (Klein et al. 2007). Vancomycin-resistant *Enterococci* (VRE) are also a concern. Currently, vancomycin is considered to be the antibiotic of last-resort. After vancomycin was first deployed in 1956, no instances of resistance were observed until 1988 (Palumbi 2001). In 2003, the percentage of *Enterococci* resistant to vancomycin was up to 28.5% (Cardo et al. 2004). Finally, many pathogens are resistant to more than one kind of antibiotic. In addition to MRSA, this has been noted in “extremely drug resistant” strains of *Mycobacterium tuberculosis*, the agent responsible for tuberculosis. Worldwide there is a concern that tuberculosis is making a comeback because of such multi-drug resistant strains, with 1.6 million deaths annually (WHO 2008). However, in the US the incidence of multi-drug resistant tuberculosis has remained low (five cases in the last 4 years) (CDC 2006; Sampathkumar 2007). In addition to these deadly antibiotic resistant pathogens, there are numerous other resistant disease-causing bacteria, ranging from deadly to nuisance.

Both the World Health Organization (WHO) and the US Center for Disease Control (CDC) have called for strategies to contain antibiotic resistance. The CDC launched a public health action plan to combat antimicrobial resistance in 1999, guided by an Interagency Task Force on Antimicrobial Resistance (CDC 2008a). Members of the task force include the US Environmental Protection Agency (US EPA), the US Department of Agriculture (USDA), the Food and Drug Administration (FDA), the US Department of Defense (DOD), and the National Institute of Health (NIH). The action plan consists of four main elements: (1) surveillance, (2) research, (3) product development, and (4) prevention and control. After almost a decade of implementation, surveillance has played an important role in documenting and understanding the problem of antibiotic resistance, as summarized above. Research and product development mainly focus on discovering new antibiotics and vaccines. The strategy with respect to prevention and control focuses largely on education of clinicians and the public. Education topics on proper use of antibiotics have included: not using antibiotics for viral infections (e.g., cold and flu), completing the full prescribed antibiotic regimen, and not taking unprescribed antibiotics. These practices are all aimed at limiting exposure of bacteria to antibiotics unless absolutely necessary, and when necessary, ensuring that all of the bacteria causing infection are eliminated so that they do not develop and pass along resistance. Proper use of antibiotics in agricultural animals is also a component of the educational campaign (CDC 2008b) and is considered a key step because the relative ease of obtaining animal antibiotics and the challenge of regulating them. What is considered proper use in animals in the US is also nuanced in comparison to what is recommended for humans, because of widely-accepted subtherapeutic use.

10.3 Mechanisms of Antibiotic Resistance

Penicillin, the first antibiotic applied in human medicine, was discovered by Sir Alexander Fleming in 1928. However, it was not until the 1940s that the drug was mass produced and widely distributed. Since then, numerous antibiotics have been discovered and applied in human medicine, including various sulfonamides, tetracyclines, macrolides, and glycopeptides. Antibiotics have proven to be so highly effective at treating bacterial (as opposed to viral) infections; such as tuberculosis, gonorrhea, syphilis, leprosy, gangrene, and pneumonia, mainly because they target aspects of the machinery for survival that are unique to bacteria and distinct from humans and animals. For example, penicillin blocks peptidoglycan production, an essential component that is unique to bacterial cell walls. Thus, antibiotics bring many of the same benefits to livestock that they do to humans, preventing and treating illness in addition to promoting overall weight gain with the net result of bringing animals to market quicker (discussed in detail in Chap. 9). However, the benefits of antibiotic use may come at a price, as consistent exposure of bacteria to antibiotics in a sense “teaches” the bacteria to overcome and resist them in the future.

Resistance of certain bacteria to penicillin and sulfonamides was observed soon after their discovery and application. While sulfonamides are synthetic, penicillin is a naturally-occurring chemical, as is the case with most antibiotics in use today. Antibiotics are produced by various fungi and certain soil-dwelling bacteria, such as *Actinomycetes*, in order to protect themselves from other microbes. In turn, bacteria have become resistant to antibiotics over time by evolving strategies to survive in their presence. Also, the bacteria and fungi that themselves produce antibiotics typically are able to resist them, analogous to producing an antidote. Some examples of such strategies include modifying the target of the antibiotic so that it is no longer effective, pumping the antibiotic out of the cell, or producing chemicals that destroy the antibiotic. Figure 10.1 provides some examples of how antibiotics work and how bacteria resist them.

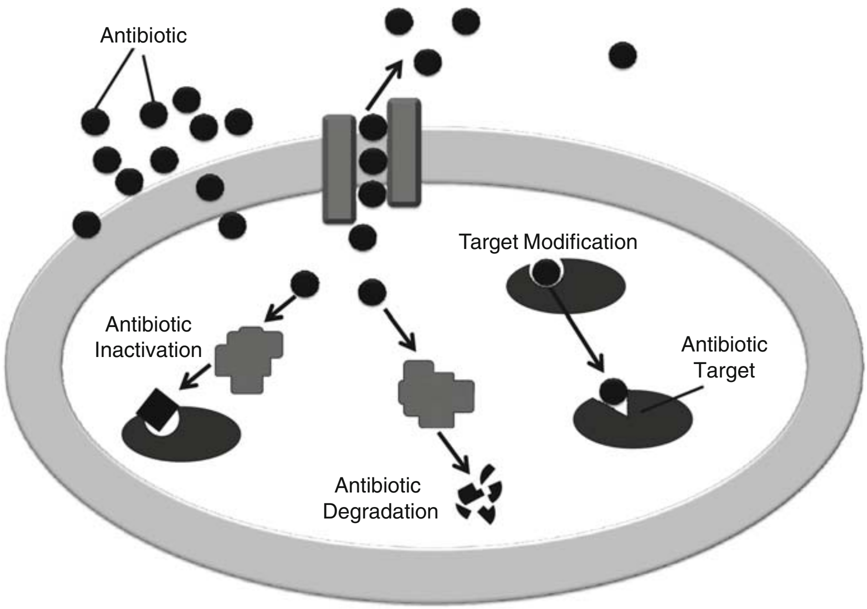


Fig. 10.1 Overview of mechanisms of antibiotic resistance (*artistic credit to Heather Storteboom*)

While antibiotic resistance is a naturally-occurring phenomenon, human activities have clearly played a role in increasing the proportion of bacteria that are resistant. This is essentially because of the principle of natural selection, commonly referred to as survival of the fittest. In the presence of antibiotics, only bacteria that are able to survive will be able to reproduce. Such bacteria carry antibiotic resistance genes (ARG), or segments of DNA that encode proteins that enable resistance and can be passed from generation to generation of bacteria. Through the widespread use of antibiotics and other factors, humans have created an environment that selects for bacteria carrying ARG. Considering that the generation time of a bacterium is very short (~30 min in *E. coli* compared to ~20 years in humans), resistance spreads very fast in bacterial populations. At the same time, it is important to realize that bacteria need several factors for basic survival, for example, food, water, nutrients, and correct environmental conditions (temperature, oxygen, pH, etc. – this varies among different bacteria). Therefore, removing an essential requirement or otherwise creating an inhospitable environment may trump the effect of an antibiotic in selecting for resistant bacteria by killing or crippling them. This provides one angle for potentially combating resistance, by killing the bacteria through appropriate waste management.

Because of a process called horizontal gene transfer, bacteria can also share ARG with other living bacteria, in addition to passing them to their offspring. Bacteria can do this in several ways. The first is transformation, in which free DNA released to the environment by one bacterium is then incorporated by another bacterium. The second is transduction, in which a virus particle that has infected a bacterium packages bacterial DNA instead of viral DNA, and then injects it into another bacterium. The third is conjugation, which is essentially a form of "mating" that takes place between certain kinds of bacterial cells through a bridge that allows DNA exchange. Integrons have recently been discovered as a fourth means of DNA exchange. Integrons are mobile pieces of DNA with the ability to capture genes, especially those encoding antibiotic resistance. Conventional wisdom has been that horizontal gene transfer takes place between bacteria of the same species; however, exchange of DNA between unrelated bacteria has now been demonstrated in several instances. In one study this was documented between two of the major antibiotic resistant pathogens of concern, *Enterococcus faecalis* and *Staphylococcus aureus* (Weigel et al. 2003). Finally, it is important to note that often ARG being exchanged are physically connected to each other along the DNA strand, thus many ARG may be passed at the same time. This is a primary cause for multi-drug resistance. Integrons in particular have been noted to confer multi-drug resistance, sometimes carrying up to 100 different ARG (Mazel 2004). Figure 10.2 provides an overview of some of the mechanisms of horizontal gene transfer.

Numerous ARG have been studied at various CAFOs. In particular, tetracycline ARG have been well-studied (Aminov et al. 2001, 2002) in part because of the common use of tetracyclines in animals and because the ecology of the genes are relatively

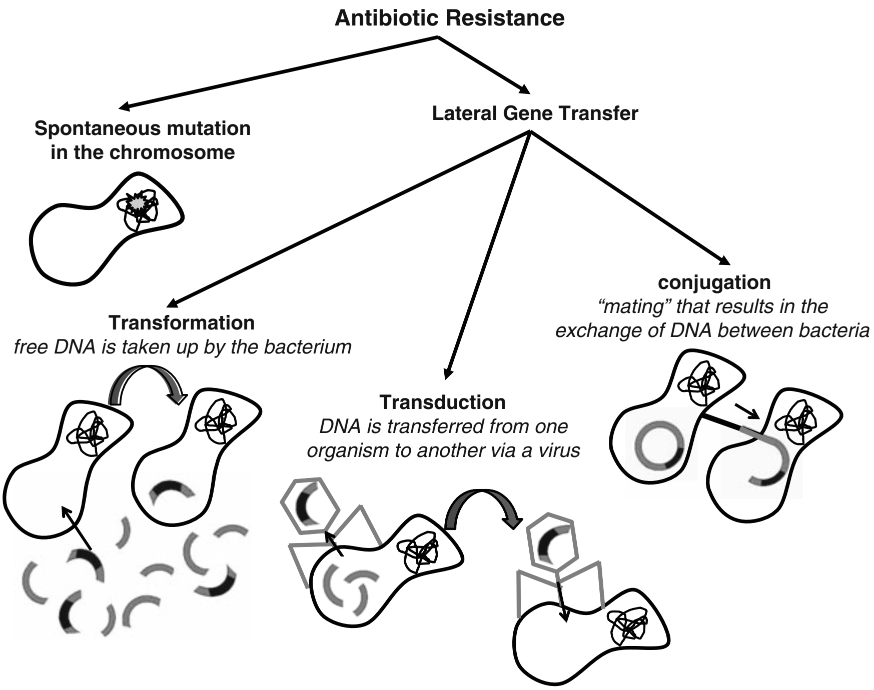


Fig. 10.2 Overview of horizontal gene transfer, mechanisms by which bacteria may share antibiotic resistance genes (ARG) (*artistic credit to Heather Storteboom*)

well-characterized. ARG associated with major human illnesses of concern, such as vanA and vanB, which cause vancomycin resistance in VRE, and mecA, which causes methicillin resistance in MRSA (Garofalo et al. 2007; Bates et al. 1994), have also been targeted. Because antibiotic resistance can be spread readily between bacteria via resistance-encoded DNA, it has been suggested that the resistance-encoded DNA itself is a pollutant and mitigation technologies should go beyond killing resistant bacteria and should further be aimed at destroying the DNA (Pruden et al. 2006).

10.4 Environmental Reservoirs and Pathways of Antibiotic Resistance

Because antibiotic resistance is naturally occurring, it is not practical or possible to eliminate all resistance. However, various human activities can amplify the level of resistance above background. Several researchers have demonstrated that ARB and ARG occur at higher levels than background in environments such as livestock lagoons, hospitals, wastewater treatment plants, and surface water adjacent to human and agricultural activity.

Figure 10.3 provides an overview of potential sources and pathways of ARB and ARG in the environment. These can be generally categorized into four broad

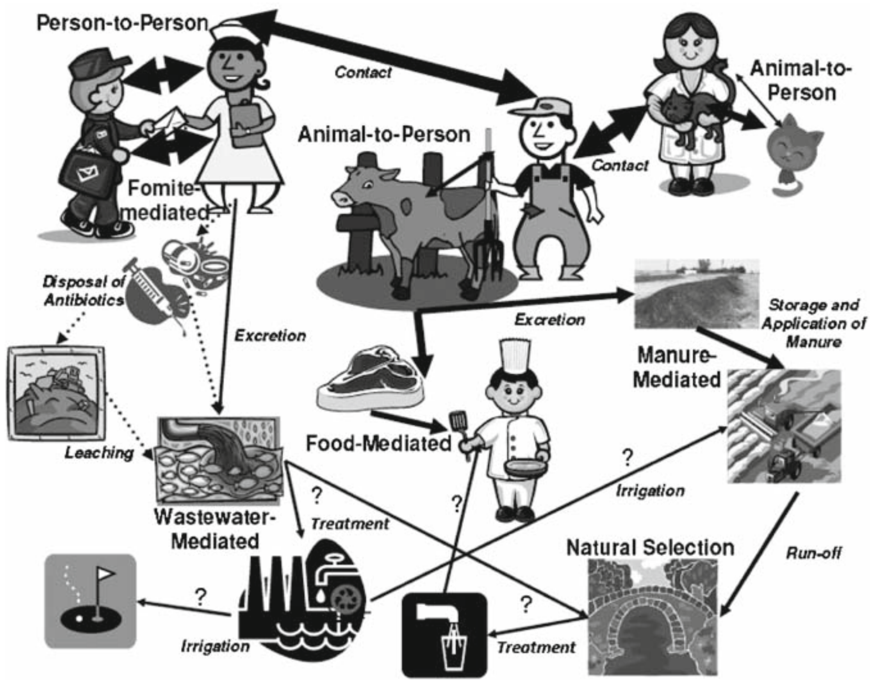


Fig. 10.3 Overview of confirmed (thicker lines) and potential (*dashed lines* = conceptual; *question marks* = resistant bacteria detected but disease transmission not confirmed) pathways of antibiotic resistance in the environment (Artistic credit to Heather Storteboom)

sources: medical care, community-acquisition, wastewater treatment, and animals/livestock. It is important to note that based on monitoring by the CDC, hospitals are by far the greatest source of resistance. People in hospitals are already weakened, so this may be one reason why there is a much higher rate of resistant infections in hospitals. Community acquisition is the next most common source and includes direct and indirect person-to-person contact. It is unknown how many people are carriers of ARB, not showing symptoms, but passing infections to others. For example, in a 2003 study of the mouths of healthy kindergarteners who had not taken antibiotics in the last 3 months showed that 97% of them carried multi-drug resistant bacteria (Ready et al. 2003). Similar parallels have been drawn to the unnaturally high concentration of animals in CAFOs and children in daycare. Thus, CAFOs are gaining attention as reservoirs of antibiotic resistance. Animal products have been documented to carry resistant bacteria (Spika et al. 1987; Belanger and Shryock 2007). Direct and indirect animal contact is also a route of exposure.

10.5 Potential Role of CAFOs in Spreading Antibiotic Resistance

The role of CAFOs in potentially spreading antibiotic resistance has been under considerable scrutiny for some time (Pew Trusts 2008; Smith et al. 2002; Teuber 2001). When an outbreak occurs, it is particularly challenging to determine the precise source of the infection. This was apparent in the recent *E. coli* outbreak associated with spinach in the US (Mukherjee et al. 2008) and the more recent outbreak of *Salmonella* associated with chili peppers (CDC 2008c). In general, various lines of evidence are followed to support a potential link. One approach is to compare the similarity of the DNA sequence (e.g., a "fingerprint") of the microorganisms responsible for the outbreak and at neighboring livestock operations. Outbreak investigations, field studies, case reports, and ecological and temporal associations also are used to trace the source of antibiotic resistant infections (Angulo et al. 2004). Based on this approach, there is a growing body of evidence that CAFOs play a significant role in contributing to the rise of antibiotic resistance.

One highly cited example in the US of CAFO-associated antibiotic use and antibiotic resistant disease in humans is that of fluoroquinolone use in poultry. The fluoroquinoline enrofloxacin, which was commonly administered under the trade name Baytril™, was banned in poultry in the US because of links to resistant *Campylobacter* infections in humans (Cimon 2001). *Campylobacter* causes food-poisoning in about one million people annually, and elevated levels of fluoroquinolone-resistant strains of *Campylobacter* have been found in meat originating from chickens administered with Baytril™. A 5 year legal battle was required to remove Baytril™ from the market (FDA 2006).

Resistant *Salmonella* infections have also been blamed on agricultural use of various antibiotics. In the UK, quinolone-resistant *S. typhimurium* DT104 infections were traced to a dairy where fluoroquinolone was used the month before the outbreak (Walker et al. 2000). Similarly, tetracycline-resistant *Salmonella* infections traced to the "top-dressing" of cattle feed with tetracycline (Holmberg et al. 1987). In California, illegal chloramphenicol use on a dairy was linked to resistant infections (Spika et al. 1987).

Arguably, resistant *Campylobacter* and *Salmonella*, which both cause gastrointestinal illness that is not typically life-threatening to healthy adults, are not major human health concerns. However, in Europe it was concluded that VRE infections in humans were linked to use of a similar glycopeptide antibiotic in broiler chickens. Vancomycin is a "last-resort" antibiotic, and therefore VRE are of significant concern to human health. Because of the link to VRE, Denmark took the lead in banning glycopeptides (Aarestrup 1995) and subsequently all subtherapeutic antibiotics use in 1999, 6 years earlier than the broader EU ban. Denmark's ban has also provided a unique opportunity to document impacts of antibiotic use in agriculture on antibiotic resistance and human health. Since institution of the ban in Denmark, a sharp decline in bacterial resistance has been observed in animal fecal material (Aarestrup et al. 2001). A significant decline has also been observed in the incidence of VRE carriage among healthy humans, though a reduction in actual VRE infections has not yet been demonstrated (Klare et al. 1999; van den Bogaard et al. 2000).

One of the most direct indicators of the impacts of antibiotic use in CAFOs on antibiotic resistance in humans is the level of resistance among individuals, such as livestock operators, working most directly with the animals. One recent study indicated that pig farmers in the Netherlands were 760 times more likely to carry MRSA on their skin than the general public (Voss et al. 2005). In Germany, shortly after nourseothricin was introduced as a growth promoter in swine, resistant determinants found in livestock operators, employees, and in neighboring communities (Hummel et al. 1986), including *Salmonella* and *Shigella* isolates (Witte et al. 2000). The first reported case of ceftriaxone-resistant *Salmonella* in the US involved the child of a veterinarian treating several herds for *Salmonella* (Fey et al. 2000). A study conducted in Ontario, Canada found a significant correlation between MRSA in pigs and pig farmers, with 24.9% of all pigs and in 20% of the farmers carrying MRSA. Interestingly, the strain detected in this study is also found in pigs in Europe and is common in US health care facilities (Khanna et al. 2007).

Thus, antibiotic have been documented to lead to increased antibiotic resistance in animals, livestock operators, and the general community. It is important to note, however, that a clear increase in resistance has not been noted in every study conducted. For example, in a recent study it was observed that commonly used ionophores did not appear to induce resistance of *Enterococci* in rumen flora (Nisbet et al. 2008). This is not necessarily surprising because the mechanism of action of ionophores is more general, acting by disrupting the ion gradient of susceptible bacteria. Thus resisting the antibiotic through modification of a single gene is unlikely, though resistance has been observed in some cases (Houlihan and Russell 2003). However, in this same study it was found that ionophore resistant bacteria were not resistant to other antibiotics. This, along with the fact that ionophores are not used in humans, provides an argument for continued ionophore use in livestock, especially considering the benefits discussed in Chap. 9.

10.6 Fate and Transport of Antibiotic Resistance in the Environment

As is the case with antibiotics, runoff and seepage are likely routes of transport of antibiotic resistance (Fig. 10.3). However, fate and transport of antibiotic resistance is more complex in that it is carried within a living host, which can grow, propagate, and spread ARG. Also, in addition to animal to human and human to human contact, bioaerosols also likely contribute to the spread of resistance from CAFOs. For example, in one study antibiotic resistant bacteria were found both in and downwind of swine CAFOs, but not upwind (Gibbs et al. 2004). Thus, we are only beginning to conceptualize the behavior of ARB and ARG in the environment. However, it has been demonstrated that agricultural activities, particularly antibiotic use, do increase resistance levels and various routes of transport have been confirmed.

Animals administered antibiotics are known to harbor elevated levels of resistant bacteria (Jackson et al. 2004; NARMS 2003) and to shed them into the environment. It has also been documented in the US that this level of carriage among livestock animals has been steadily increasing. For example, ceftiofur resistance among *Salmonella* carried by humans and livestock, cattle in particular, have steadily increased (NARMS 2003). Molecular based techniques targeting ARG directly, such as genetic fingerprinting and quantitative polymerase chain reaction (Q-PCR), provide a means to asses the agricultural impacts in terms of observed resistance in the environment. For example, a study of the Cache La Poudre (Poudre) River in northern Colorado demonstrated that there was a relationship between urban and agricultural land-use and quantities of tetracycline and sulfonamide ARG in the river sediments (Pruden et al. 2006). An advantage of this study was that the Poudre River is relatively pristine in origin, with its headwaters in the Rocky Mountains. Thus it was possible to measure background levels of naturally-occurring resistance and contrast these with the higher levels of ARGs observed downstream. While it could be determined that both agricultural and urban activities contributed to the levels of resistance observed, quantifying relative inputs from the two sources is more challenging and will likely require molecular fingerprinting of ARGs.

Animal waste is typically treated by lagoon and/or composting, and the residuals are land-applied. Several studies have demonstrated the presence of ARB and ARG in lagoon water and in the groundwater beneath (Koike et al. 2007; Pei et al. 2007; Peak et al. 2007; Smith et al. 2004; Chee-Sanford et al. 2001). ARG are also present in compost (Storteboom et al. 2007). Thus, runoff and seepage are both likely routes of spreading ARB and ARG off-farm. At the same time, the "treatment" processes themselves may act to either decrease or increase antibiotic resistance (Pei et al. 2007; Storteboom et al. 2007). Again, this is because of the nature of the "contaminant" being a living host which will be selected for or against under certain environmental conditions, depending on its ecology.

The relationship between concentrations of antibiotics in the environment and the level of resistance is not well-understood. Intuitively, it would seem that there would be a direct correlation. Such a correlation was identified by Peak et al. (2007) in a recent survey of cattle feedlot lagoons in Kansas. However, a correlation was not found between antibiotics and ARG in river sediments (Pei et al. 2006). Inconsistency of this correlation may be due to a variety of factors. For example, resistant bacteria may initially be selected for in the presence of antibiotics, but then be transported elsewhere and/or harbor resistance long after the antibiotic has been removed or has degraded. Also, because ARG may be physically linked to other genes, such as metal resistance, other environmental factors (e.g., elevated heavy metals) may also select for antibiotic resistance.

10.7 Summary

Thus, it has been demonstrated that antibiotic use in CAFOs is associated with elevated antibiotic resistance in animals, humans, and the general environment. However, it is difficult to quantify the exact contribution of antibiotic use in CAFOs to observed antibiotic resistant disease in humans. Characterizing the fate and transport of ARB and ARG is also challenging due to the nature of the contaminant being harbored within a living host. With these thoughts in mind, potential best management practices will be discussed in Chap. 15.

References

Aarestrup FM (1995) Occurrence of glycopeptide resistance among Enterococcus faecium isolates from conventional and ecological poultry farms. Microb Drug Resist 1:255–257

Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F (2001) Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal Enterococci from food animals in Denmark. Antimicrob Agents Chemother 45:2054–2059

Aminov RI, Garrigues-Jeanjean N, Mackie RI (2001) Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl Environ Microbiol 67:22–32

Aminov RI, Chee-Sanford JC, Garrigues N, Teferedegne B, Krapac IJ, White BA, Mackie RI (2002) Development, validation, and application of PCR primers for detection of tetracycline efflux genes of gram-negative bacteria. Appl Environ Microbiol 68:1786–1793

Angulo FJ, Nargund VN, Chiller TC (2004) Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med 51:374–379

Bates J, Jordens JZ, Griffiths DT (1994) Farm-animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemother 34:507–514

Belanger AE, Shryock TR (2007) Macrolide-resistant Campylobacter: the meat of the matter. J Antimicrob Chemother 60:715–723

Cardo D, Horan T, Andrus M, Dembinski M, Edwards J, Peavy G, Tolson J, Wagner D (2004) National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control 32:470–485

Center for Disease Control (CDC) (2006) Annual report on progress on “A public health action plan to combat antimicrobial resistance”. http://www.cdc.gov/drugresistance/actionplan/2006report/index.htm [Web link verified June 12, 2008]

Center for Disease Control (CDC) (2008a) A public health action plan to combat antimicrobial resistance. http://www.cdc.gov/drugresistance/actionplan/index.htm [Web link verified June 12, 2008]

Center for Disease Control (CDC) (2008b) Get smart: know when antibiotics work on the farm. Educational activities to promote appropriate use of antimicrobial agents in animals. http://www.cdc.gov/narms/get_smart.htm [Web link verified June 12, 2008]

Center for Disease Control (CDC) (2008c) Investigation of outbreak of infections caused by *Salmonella* Saintpaul. http://www.cdc.gov/salmonella/saintpaul/ [Web link verified June 12, 2008]

Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI (2001) Occurrence and diversity of tetracycline resistance genes in lagoons. Appl Environ Microbiol 67:1494–1502

Cimon M (2001) FDA planning to halt Ag use of two antibiotics. ASM News 67:9–10

Fey P, Safranek T, Rupp M, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs, SH (2000) Ceftriax-one-resistant Salmonella infection acquired by a child from cattle. N Engl J Med 342:1242–1249

Garofalo C, Vignaroli C, Zandri G, Aquilanti L, Bordoni D, Osimani A, Clementi F, Biavasco F (2007) Direct detection of antibiotic resistance genes in specimens of chicken and pork meat. Int J Food Microbiol 113:75–83

Gibbs SG, Green CF, Tarwater PM, Scarpino PV (2004) Airborne antibiotic resistant and nonresistant bacteria and fungi recovered from two swine herd confined animal feeding operations. J Occup Environ Hyg 1:699–706

Holmberg, SD, Solomon, SL, Blake PA (1987) Health and economic impacts of antimicrobial resistance. Rev Infect Dis 9:1065–1078

Houlihan AJ, Russell JB (2003) The susceptibility of ionophore-resistant Clostridium aminophilum F to other antibiotics. J Antimicrob Chemother 52:623–628

Hummel R, Tschape H, Witte W (1986) Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry. J Basic Microbiol 26:461–466

Jackson CR, Fedorka-Cray PJ, Barrett JB, Ladely SR (2004) Effects of tylosin use on erythromycin resistance in enterococci isolated from swine. Appl Environ Microbiol 70:4205–4210

Khanna T, Friendship R, Dewey C, Weese JS (2007) Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol 128:298–303

Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W (1999) Decreased incidence of van A-type vancomycin-resistant *enterococci* isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist 5:45–52

Klein E, Smith DL, Laxminarayan R (2007) Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999–2005. Emerg Infect Dis 13:1840–1846

Klevens RM, Edwards JR, Richards CL, Horan TC, Gaynes RP, Pollock DA, Cardo DM (2007) Estimating health care-associated infections and deaths in US hospitals, 2002. Public Health Rep 122:160–166

Koike S, Krapac IG, Oliver HD, Yannarell AC, Chee-Sanford JC, Aminov RI, Mackie RI (2007) Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Appl Environ Microbiol 73:4813–4823

Leeb M (2004) Antibiotics: a shot in the arm. Nature 431:892–893

Mazel D (2004) Integrons and the origin of antibiotic resistance gene cassettes. ASM News 70:520

Mukherjee A, Mammel MK, LeClerc JE, Cebula TA (2008) Altered utilization of N-acetyl-D-galactosamine by Escherichia coli O157: H7 from the 2006 spinach outbreak. J Bacteriol 190:1710–1717

National Antimicrobial Resistance Monitoring System (NARMS) (2003) Executive Report on Enteric Bacteria. Available online at http://www.fda.gov/cvm/Documents/NARMSExecSum03.pdf [verified June 12, 2008]

Nisbet DJ, Callaway TR, Edrington TS, Anderson RC, Poole TL (2008) Effects of lonophores on Enterococcus faecalis and E. faecium growth in pure and mixed ruminal culture. Foodborne Pathog Dis 5:193–198

Palumbi SR (2001) Humans as the world's greatest evolutionary force. Science 293:1786–1790

Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW (2007) Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. Environ Microbiol 9:143–151

Pei R, Kim SC, Carlson KH, Pruden A (2006) Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). Water Res 40:2427–2435

Pei R, Cha J-M, Carlson KH, Pruden A (2007) Biological treatment of antibiotic resistance fenes (ARG) in dairy lagoon water. Environ Sci Technol 41:5108–5113

Pew Charitable Trusts and Johns Hopkins Bloomberg School of Public Health (2008) Putting Meat on the Table: Industrial Farm Animal Production in America. Available online at http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Reports/Industrial_Agriculture/PCIFAP_FINAL.pdf (verified June 12, 2008)

Pruden A, Pei R, Storteboom HN, Carlson KH (2006) Antibiotic resistance genes (ARG) as emerging contaminants: studies in Northeastern Colorado. Environ Sci Technol 40:7445–7450

Ready D, Bedi R, Spratt DA, Mullany P, Wilson M (2003) Prevalence, proportions, and identities of antibiotic-resistant bacteria in the oral microflora of healthy children. Microb Drug Resist 9:367–372

Sampathkumar P (2007) Dealing with threat of drug-resistant tuberculosis: background information for interpreting the Andrew Speaker and related cases. Mayo Clin Proc 82:799–802

Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris Jr JG (2002) Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci USA 99:6434–6439

Smith MS, Yang RK, Knapp CW, Niu YF, Peak N, Hanfelt MM, Galland JC, Graham DW (2004) Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR Appl Environ Microbiol 70:7372–7377

Spika J, Waterman S, Hoo G, St Louis ME, Pacer RE, James SM, Bissett ML, Mayer LW, Chiu JY, Hall, B (1987) Chloramphenicol-resistant *Salmonella* Newport traced through hamburger to dairy farms: a major persisting source of human salmonellosis in California. N Engl J Med 316:565–570

Storteboom HN, Kim SC, Doesken K, Davis J, Carlson KH, Pruden A (2007) Response of antibiotics and resistance genes to high-intensity and low-intensity manure management. J Environ Qual 36:1695–1703

Teuber M (2001) Veterinary use and antibiotic resistance. Curr Opin Microbiol 4:493–499

US Food and Drug Administration (FDA) (2006) Docket No 200 N-1571: withdrawal of approval of the new animal drug application for enrofloxacin in poultry. Department of Health and Human Services

van den Bogaard AE, Bruinsma N, Stobberingh EE (2000) The effect of banning avoparcin on VRE carriage in The Netherlands. J Antimicrob Chemother 46:146–148

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005) Methicillin-resistant Staphylococcus aureus in pig farming. Emerg Infect Dis 11:1965–1966

Walker RA, Lawson AJ, Lindsay EA, Ward LR, Wright A, Bolton FJ, Wareing DRA, Corkish JD, Davies RH, Threlfall EJ (2000) Decreased susceptibility to ciprofloxacin in outbreak-associated multiresistant *Salmonella typhimurium* DT104. Vet Rec 147:395–396

Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC (2003) Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. Science 302:1569–1571

Witte W, Tschape H, Klare I, Werner G (2000) Antibiotics in animal feed. Acta Vet Scand 93:37–45

World Health Organization (WHO) (2008) A world free of TB. http://www.who.int/tb/en/ [Weblink verified June 12, 2008]

Chapter 11
Effects of Steroid Hormones on Aquatic and Soil Organisms

Laurence Shore

Abstract The naturally occurring steroid hormones are evolutionary old compounds and have documented effects in most phyla. The problem for environmental effects is therefore a matter of determining which effects may have an environmental impact at concentrations released from CAFOs. Of primary concern are the suspected effects on fish reproduction. The effects of steroids on soil organisms may actually increase biomass as steroids can serve as a carbon source.

11.1 Introduction

Since the original observations of Sumpter and Jobling (Purdam et al. 1994; Sumpter 1995; Sumpter and Jobling 1995), the presence of fish displaying intersex or sex reversal downstream from STWPs has been observed worldwide (Jobling et al. 1998). However, the agent causing the effect is most probably ethinylestradiol (EE) which is effective in ng/l amounts. Indeed of all the hundreds of pharmaceuticals and personal care products (PCPPs) which have been detected in surface waters, EE is the only one for which an adverse environmental effect has been clearly indicated (Huggert et al. 2004; Webb 2004). In addition, since the original observations of Howell et al. (1980) compounds (most likely androstenedione) produced as metabolites in pulp mill effluent has been well documented to masculinize fish. There is scant information of the effects of animal excreta on fish or other animals in the absence of EE (see Chap. 13). However, since the physiologically active steroid hormones have been studied intensively for the last 50 years, there is a large amount of information about the effects of natural and synthetic hormones on various organisms and the problem is more of determining the relevance of the literature to possible environmental effects than understanding potential modes of action.

L. Shore (✉)
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute,
POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_11,

11.2 Response of Aquatic Organisms to Hormones

The parameters of steroid hormone response most commonly used are vitellogenin (Vtg), intersex, secondary sexual characteristics and sex ratio (Table 11.1). Molecular biology techniques for studying up and down regulation by estradiol have not been widely adopted due to the lack of extensive fish genomic libraries although this would give information on E effects not directly related to egg production (Denslow et al. 2001). Vitellogenin is the egg protein produced in the liver of the female fish and is usually not present in the male. Although the presence of Vtg is a good environmental marker, it is not necessarily correlated with any physiological effect in male fish reproduction. Older literature reported effects on susceptibility to infection and mortality but these parameters have not been measured in recent environmental literature (Table 11.1). Fish are most sensitive to E2 in early post-hatching period and much higher concentrations are needed for effects on adult fish (Krisfalusi and Nagler 2000). Environmental studies are complicated by the fact that the fish themselves produce measurable amounts of testosterone and estradiol (3–15 ng/l) (Kramer et al. 1998; Shore et al. 1993, 2007). Furthermore fish eat other fish with gonads (about 3% body wt) which contain hormones, e.g., the yearling carp ovary contains 200 ng of E1+E2 (Naber M, Shore LS, personal observations) and the testes contains 140-ng 11-ketotestosterone and 40-ng T (Degani et al. 1998). It would therefore appear that predator fish should have ample mechanisms to inactivate or excrete natural steroid hormones. However, there is evidence that the trout may bioaccumulate the steroid hormones, at least as evidenced by the bile content (Larsson et al. 1999).

Although E2 is considered the most potent estrogen in receptor assays and mouse uterine test, E1 and even estriol may be more potent in causing changes in sex ratio in *Oryzias latipes* (Metcalfe et al. 2001). The effects of estrone and estradiol may be additive (Panter et al. 1998; Routledge et al. 1998).

While the Vtg responses in cyprinoid and non-cyprinoid fish are in the range of 10 ng/l, pheromone responses have been reported for a number of steroids in the ng/l range (Chap. 2, Section 2.5). This makes it difficult to interpret the environmental impact of the pheromones as levels reported are above those which are commonly found in the environment. Changing sex with steroidal compounds is a routine procedure in aquaculture. These compounds are considered separately in Chap. 12.

11.3 Response of Soil Organisms to Steroid Hormones

The response of organisms living at or below the trophic level of soil organisms, downstream from vertebrates, is difficult to assess since many of these organisms do not have recognizable hormone receptors although they appear to have steroid regulated reproductive cycles (Oehlmann and Schulte-Oehlmann 2003). As discussed in the chapter 8, most of the soil biomass consists of nematodes, fungi and bacteria although microarthropods are present.

Table 11.1 Effects of steroid hormones on (a) Cyprinoid fish and (b) non-Cyprinoid

Species	Concentrations	Effects		References
(a) Cyprinoid fish				
Cyprinodon variegatus	NOEC 20 ng E2/l; LOEC 65 ng/l	Vtg – 13 days		Folmar et al. (2000)
Carassius auratus	50-mg E2 implant	Parasitaema Mortality	↑	Wang and Belosevic (1994)
Danio rerio	$E2_{10}$ 15 ng/l; E_{50} 41 ng/l; E_{90} 67 ng/l; NOEC 13 ng E2/l; LOEC 21 ng/l	Vitellogenin – 8 days	↑	Rose et al. (2000)
Danio rerio	NOEC 5 ng E2/L; LOEC 25 ng/L ; Significant 100 ng	Vitellogenin – 21 days		Brian et al. (2005)
Danio rerio	5 E2 ng/l	Decrease in viable eggs – 210 days Lower 11 – ketotestosterone		Nash et al. (2004)
Danio rerio	$E2_{10}$ 57 ng/l	Ovarian somatic index – 21 days juvenile		Van den Belt et al. (2004)
Danio rerio	$E1_{10}$ 195 ng/l	Ovarian somatic index – 21 days juvenile		Van den Belt et al. (2004)
Pimephles promelas	NOEC 5 ng E2/l; LOEC 7.5 ng/l; ED_{50} 25 ng/l	Vitellogenin – 14 days		Brian et al. (2005)
Pimephles promelas	NOEC 10 ng E2/l; LOEC 32 ng/l; Significance 100 ng	Vitellogenin – 21 days		Panter et al. (1998)
Pimephles promelas	NOEC 361 ng E1/l; LOEC 781 ng/l	21 days Hatching success, egg batch size Mortality in males; spawning frequency		Thorpe et al. (2003)
Rutilus rutilus	NOEC 10 ng E2/l; LOEC 100 ng/l	Vitellogenin – 21 days		Routledge et al. (1998)

(continued)

Table 11.1 (continued)

Species	Concentrations	Effects		References
(b) Non-Cyprinoid fish				
Chrysophrys majors paridae	3 mg E2/kg in feed for 30 days	HIS	↑	Woo et al. (1993)
		Vitellogenin	↑	
		Lipid	↑	
		Cholesterol	↑	
		Amino acids	↑	
Pagrus major (*previously Chrysophrys major*)	3 mg T/kg in feed for 30 days	HIS	–	Woo et al. (1993)
		GIS	–	
		Vitellogenin	–	
		Lipid	↑	
		Cholesterol	↑	
		Amino acids	↑	
Oncorhynchus masou *Oncorhynchus keta*	0.5–1.0 µg E2/l	18 days Intersex Mortality	50%	Nakamura (1984)
Oncorhynchus mykiss	NOEC 1 ng E2/l; LOEC 10 ng/l; NOEC 25 ng E1/l; LOEC 50 ng/l	Vtg – 21 days	40%	Routledge et al. (1998)
Oryzias latipes	LOEC 10 ng E2/l; NOEC 1 ng E1/l; LOEC 10 ng/l	Intersex – 100 days		Metcalfe et al. (2001)
Oryzias latipes	100 µg T/l for 6 days	Intersex		Koger et al. (2000)

NOEC no observable effect concentration, *LOEC* lowest observable effect concentration, ED_{50} Effective dose 50% level

11.3.1 Nematodes

The free living soil nematode, *Caenorhabditis elegans*, is one of the basic tools of molecular biology. At least for *C. elegans*, estrogen receptors and estrogen responsive elements (ERE) have been reported but these receptors are not related to the vertebrate receptors (Mimoto et al. 2007). Furthermore, all of the natural steroids examined (P, E2, T) up or down regulate numerous genes as shown in microarrays (Custodia et al. 2001). Nematodes require sterols such as cholesterol for proper growth and reproduction and steroids can apparently also be utilized (Höss and Weltje 2007). In general, the effects of natural steroids at environmental levels are positive in increasing growth and fecundity (Table 11.2) and the amounts required to see an effect on growth and reproduction are well above environmental levels

11.3.2 Fungi

As mentioned in Chap. 2.7, the steroids may be toxic to fungi and the fungi have developed protective mechanisms against the antifungal sterols produced by some plants (Breskvar et al. 1995; Vitas et al. 1999). In addition, some of the parasitic fungi have developed relationships with their host's hormones (Clemons et al. 1989). Chun et al. (2006) reported that addition of 2 mg/kg of E2 to soil, which changed the nature of the bacteria community, had no effect on fungi. Similarly, as noted in chapter 8, application of sludge with fecal steroids had no effect on the fungal biomass.

Table 11.2 Effects of steroidal hormones on soil nematodes

Species	Concentrations	Effects	References
Caenorhabditis elegans	E2 NOEL 2.7 ng/l 6 days, LOEL 27 ng/l Max 270 ng/l	Increased germ cell no.	Hoshi et al. (2003)
C. elegans	E2, T, methyltestosterone NOEL 136 μg/l	Fecundity	Tominaga et al. (2002)
	E2 LOEL 272 ng/l	VTG	Custodia et al. (2001)
Cephalobus sp	E2 LOEL 1 μg/l T LOEL 10 μg/l	Increase body length	Thong and Webster (1971)
C. elegans *Panagrellus redivivus*	E2, T, Methyltestosterone NOEL 100 mg/l P LOEL 25 mg/l	No effect No. of offsprings reduced	Dropkin et al. (1971)

11.3.3 Bacteria

Chun et al. (2005) using 2 mg/kg of E2 initially reported that estradiol increased biomass and that antibiotics reduced this effect. In a subsequent publication (Chun et al. 2006), the authors were unable to confirm their hypothesis, but did show changes in microbial community, in particular an increase the markers for the beta proteobacteria. Some beta proteobacteria, e.g., *Comamonas testosteroni*, can metabolize a wide variety of aromatic compounds and steroids as a carbon source and this activity is part of the normal pathway of soil degradation of cellulose (Möbus et al. 1997).

The *Rhizobia* are bacteria that infiltrate the roots of legume plants and create nodules. This is a symbiotic relationship which allows the plants to nitrify elemental nitrogen. The specific *Rhizobium* species find the correct legume plant by a series of chemical signals. The initial signal is activation of the *NodD* gene in the rhizobium. This gene is activated by a particular phytoestrogen produced by each legume species. Application of estrogen in low concentrations (both E1 and E2, 10 ng/l) results in alfalfa plants with larger nodules and more vegetative growth (Shore et al. 1992, 1995). However, at the same time the plants produce more phytoestrogens and the concentrations may rise to levels that can interfere with reproduction in the cattle which ingest the plants. This rise in phytoestrogens in alfalfa was observed under field conditions following irrigation with treated sewage water. However, P, T, E1, E2, E3 had no significant effect on *NodD* (Shore et al. 1993; Fox et al. 2004). In any event, the evidence indicates that estrogen and testosterone in the soil do not accumulate in plants at environmental concentrations (Shore et al. 1993).

11.3.4 Gastropod, Isopods, Annelids

The mollusks and the annelids live in or on the soil surface and can bioaccumulate many anthropomorphic compounds. Unlike a least one hermaphroditic snail where P, E and T (Alon et al. 2007) have been shown to be endogenous and correlated with reproductive status, the earthworm does not have endogenous E2 (Markman et al. 2007). Although the effects of endocrine disruptors on imposex have been characterized in fresh water snails (Oehlmann and Schulte-Oehlmann 2003; Oehlmann et al. 2007), there is no literature on the effects of steroids on terrestrial gastropods or isopods which ingest soil or utilize soil water.

11.4 Summary

The LOEL for fish species for an increase in vitellogenin by estrogen (estrone or estradiol) is 10 ng/l for 21 days. The level required to change the sex of a fish is 10 ng/l for 100 days. The LOEL for causing 40% mortality is 500 ng/l. Testosterone has no reported physiological effects below μg/l. Generally levels of estrogen or testosterone above 50 ng/l have not been found in any aquatic environment with the exception of the USGS study which claimed maximums of 200 ng/l for estrogen, 112-ng/l estrone and 214 ng/l of testosterone. However, species specific pheromone effects have been reported for testosterone and estrogen in 1 ng/l range. For fungi, nematodes and gastropods, the effect of environmental concentrations, if any, would be positive. For bacteria, both estrone and estradiol at applied at 10 ng/l increased nodule size, phytoestrogen content and increased vegetative growth in alfalfa which was mediated through the *Rhizobium*.

References

Alon G, Shore LS, Steinberger Y (2007) Correlation between levels of sex hormones (progesterone, testosterone, estrogen) and ecophysiological behavior stages in two species of desert snails (*Sphincterochila zonata* and *Sphincterochila prophetarum*) in the northern Negev Desert. Gen Comp Endo 151:122–127

Breskvar K, Ferenčak Z, Hudnik-Plevnik T (1995) The role of cytochrome $P450_{11\alpha}$ in detoxification of steroids in the filamentous fungus *Rhizopus nigricans*. J Steroid Biochem Mol Biol 52:271–275

Brian JV, Harris CA, Scholze M, Backhaus T, Booy P, Lamoree M, Pojana G, Jonkers N, Runnalls T, Bonfà A, Marcomini A, Sumpter JP (2005) Prediction of the response of freshwater fish to a mixture of estrogenic chemicals. Environ Health Perspect 113:721–728

Chun S, Lee J, Geyer R, White DC, Raman DR (2005) Effect of agricultural antibiotics on the persistence and transformation of 17*β*-estradiol in a Sequatchie loam. J Environ Sci Health B 40:741–751

Chun S, Lee J, Radosevich M, White DC, Geyer RJ (2006) Influence of agricultural antibiotics and 17*β*-estradiol on the microbial community of soil. Environ Sci Health B 41:923–935

Clemons KV, Stover EP, Schar G, Stathis PA, Chan K, Tokes L, Stevens DA, Feldman D (1989) Steroid metabolism as a mechanism of escape from progesterone-mediated growth inhibition in *Trichophyton mentagrophytes*. J Biol Chem 264:11186–11192

Custodia N, Won SJ, Novillo A, Wieland M, Li C, Callard IP (2001) *Caenorhabditis elegans* as an environmental monitor using DNA microarray analysis. Ann NY Acad Sci 948:32–42

Degani G, Boker R, Jackson K (1998) Growth hormone, sexual maturity and steroids in male carp (*Cyprinus carpio*). Comp Biochem Physiol C 120:433–440

Denslow ND, Leeb HS, Bowmanc CJ, Hemmerd MJ, Folmard LC (2001) Multiple responses in gene expression in fish treated with estrogen. Comp Biochem Physiol B 129:277–282

Dropkin VH, Lower WR, Acedo J (1971) Growth inhibition of *Caenorhabditis elegans* and *Panagrellus redivivus* by selected mammalian and insect hormones. J Nematol 3:349–355

Folmar LC, Hemmer M, Hemmer R, Bowman C, Kroll K, Denslow ND (2000) Comparative estrogenicity of estradiol, ethynylestradiol and diethylstilbestrol in an in vivo, male sheepshead minnow (*Cyprinodon variegatus*), vitellogenin bioassay. Aquat Toxicol 49:77–88

Fox JE, Starcevic M, Jones PE, Burow ME, McLachlan JA (2004) Phytoestrogen signaling and symbiotic gene activation are disrupted by endocrine-disrupting chemicals. Environ Health Perspect 112:672–677

Hoshi H, Kamata Y, Uemura T (2003) Effects of 17β-estradiol, bisphenol A and tributyltin chloride on germ cells of *Caenorhabditis elegans*. J Vet Med Sci 65:881–885

Höss S, Weltje L (2007) Endocrine disruption in nematodes: effects and mechanisms Ecotoxicology 16:15–28

Howell WM, Black DA, Bortone SA (1980) Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally-induced masculinization. Copeia 1980:676–681

Huggert DB, Ericson JF, Cook JC, Williams RT (2004) In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn. Springer-Verlag, Heidelberg Berlin pp 373–386

Jobling S, Nolan M, Tyler CR, Brighty GC, Sumpter JP (1998) Widespread sexual disruption in wild fish. Environ Sci Technol 32:2498–2506

Koger CS, Teh SJ, Hinton DE (2000) Determining the sensitive developmental stages of intersex induction in medaka (*Oryzias latipes*) exposed to 17ß-estradiol or testosterone. Marine Environ Res 50:201–206

Kramer VJ, Miles-Richardson S, Pierens SL, Giesy GP (1998) Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (*Pimephales promelas*) exposed to waterborne 17β-estradiol. Aquat Toxicol 40:335–360

Krisfalusi M, Nagler JJ (2000) Induction of gonadal intersex in genotypic male rainbow trout (*Oncorhynchus mykiss*) embryos following immersion in estradiol-17ß. Mol Reprod Dev 56:495–501

Larsson DGJ, Erici MA, Parkkonen J, Pettersson M, Berg AH, Olsson PE, Forlin L (1999) Ethinyloestradiol - An undesired fish contraceptive? Aquat Toxicol 45:91–97

Markman S, Guschina IA, Barnsley S, Buchanan KL, Pascoe D, Müller CT (2007) Endocrine disrupting chemicals accumulate in earthworms exposed to sewage effluent. Chemosphere 70:119–125

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vivo* assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 20:297–308

Mimoto A, Fujii M, Usami M, Shimamura M, Hirabayashi N, Kaneko T, Sasagawa N, Ishiura S (2007) Identification of an estrogenic hormone receptor in Caenorhabditis elegans. Biochem Biophy Res Commun 364:883–888

Möbus E, Jahn M, Schmid R, Jahn D, Maser E (1997) Testosterone-regulated expression of enzymes involved in steroid and aromatic hydrocarbon catabolism in *Comamonas testosteroni*. J Bacteriol 179:5951–5955

Nakamura M (1984) Effects of estradiol-17β on gonadal sex differentiation in two species of salmonids, the masu salmon, *Oncorhynchus masou*, and the chum salmon. O. *keta*. Aquaculture 43:83–90

Nash JP, Kime DE, Van der Ven LTM, Wester PW, Brion F, Maack G (2004) Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ Health Perspect 112:1725–1733

Oehlmann J, Schulte-Oehlmann U (2003) Endocrine disruption in invertebrates. Pure Appl Chem 75:2207–2218

Oehlmann J, Di Benedetto P, Tillmann M, Duft M, Oetken M, Schulte-Oehlmann U (2007) Endocrine disruption in prosobranch molluscs: evidence and ecological relevance. Ecotoxicology 16:29–43

Panter GH, Thompson RS, Sumpter JP (1998) Adverse reproductive effects in male fathead minnows (*Pimephles promelas*) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. Aquat Toxicol 42:243–253

Purdam CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP (1994) Estrogenic effects of effluent from sewage treatment works. Chem Ecol 8:275–285

Rose J, Holbech H, Lindholst C, Norum U, Povlsen A, Korsgaard B, Bjerregaard P (2000) Vitellogenin induction by 17β-estradiol and 17α-ethinylestradiol in male zebrafish (*Danio rerio*). Comp Biochem Physiol C 131:531–539

Routledge EJ, Sheahan D, Desbrow GC, Brighty M, Waldock M, Sumpter JP (1998) Identification of estrogenic chemicals in STW effluent. 2. *In vivo* responses in trout and roach. Environ Sci Technol 32:1559–1565

Shore LS, Kapulnik Y, Ben-Dov B, Fridman Y, Wininger S, Shemesh M (1992) Effects of estrone and 17ß-estradiol on vegetative growth of *Medicago sativa*. Physiol Plant 84:217–222

Shore LS, Gurevich M, Shemesh M, (1993) Estrogen as an environmental pollutant. Bull Environ Contam Toxicol 51:361–366

Shore LS, Kapulnik Y, Gurevich M, Wininger S, Badamy H, Shemesh M (1995) Induction of phytoestrogens production in *Medicago sativa* leaves by irrigation with sewage water. Environ Exp Bot 35:363–369

Shore LS, Cuneah O, Barel-Cohen K (2007) Concentrations of androstenedione and testosterone in manure, effluent, soil and surface water. In:Proceedings of the 6th international conference on pharmaceuticals and EDC chemicals in water. http://ngwa.confex.com/ngwa/pharm6/techprogram/S2007.HTM

Sumpter JP (1995) Feminized responses in fish to environmental estrogens. *Toxicol Lett* 82(83):737–742

Sumpter JP, Jobling S (1995) Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect 103:173–178

Thong CHS, Webster JM (1971) The effect of gonadotrophins on the in vitro growth of the free-living nematode *Cephalobus* sp. Bastian. Can J Zool 49:1059–1061

Thorpe KL, Benstead R, Hutchinson TH, Cummings RI, Tyler CR (2003) Reproductive effects of exposure to oestrone in the fathead minnow. Fish Physiol Biochem 28:451–452

Tominaga N, Tomoeda M, Kohra S, Takao Y, Nagae M, Ueda K, Ishibashi H, Kai T, Arizono K (2002) A convenient sublethal assay of alkylphenol and organotin compounds using the nematode *Caenorhabditis elegans*. J Health Sci 48:555–559

Van den Belt K, Berckmans P, Vangenechten C, Verheyen R, Witters H (2004) Comparative study on the in vitro/in vivo estrogenic potencies of 17β-estradiol, estrone, 17α-ethynylestradiol and nonylphenol. Aquat Toxicol 66:183–195

Vitas M, Smith KE, Plavec J, Kesselmeier J, Pajič T, Ferlan A, Žigon D, Kelly SL, Komel R (1999) Induction of steroidal hydroxylase activity by plant defence compounds in the filamentous fungus *Cochliobolus lunatus*. Chemosphere 38:853–863

Wang R, Belosevic M (1994) Estradiol increases susceptibility of goldfish to *Trypanosoma danilewskyi*. Dev Comp Immunol 18:377–387

Webb SF (2004) A data based perspective on the environmental risk assessment of human pharmaceuticals III- indirect human exposure. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn. Springer-Verlag, Heidelberg Berlin pp 373–386

Woo NYS, Chung ASB, Ng TB (1993) Influence of oral administration of estradiol-17β and testosterone on growth, digestion, food conservation and metabolism in the underyearling red sea bream, *Chrysophrys major*. Fish Physiol Biochem 10:377–387

Chapter 12
Organic Compounds used in Aquaculture

Thomas Heberer

Abstract The transport patterns and potential direct or indirect effects on the environment or even on human health of agents used in aquaculture like antibiotics, triphenylmethane dyes and hormones used to modify sex ratios have barely been defined. Even at trace levels, hormones may influence the endocrine systems of non-target organisms due to their high biological low-dose activity. Antibiotics are often also active at low concentrations but by their nature do not directly affect benthic species. Nevertheless, prophylactic use of antimicrobials in aquacultures may result both in environmental or human health risks. Potential risks are primarily the direct or indirect transfer of resistant bacteria generated by antibiotic treatment and the development of reservoirs of transferable resistance genes in bacteria in aquatic environments. Another important issue is the occurrence of veterinary drugs in fish and fish products intended for human consumption. Illegal uses, and also environmental background contaminations from legal sources, may lead to residues that might be unacceptable with regard to consumer health for toxicological reasons or simply for legal reasons such as violation of zero tolerances.

12.1 Introduction

"Aquaculture is developing, expanding and intensifying in almost all regions of the world" as it was stated in a report recently published by the Fisheries and Aquaculture Department of the Food and Agriculture Organization (FAO) of the United Nations (FAO 2006). While global demand for aquatic food products is increasing, the production from capture fisheries has leveled off and most of the main fishing areas have reached their maximum potential. Thus, the FAO (2006)

T. Heberer (✉)
Oldenburg Food Institute, Lower Saxony Federal State Office of Consumer Protection and Food Safety, Martin-Niemöller-Straße 2, 26133, Oldenburg, Germany
e-mail: Thomas.heberer@laves.niedersachsen.de

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_12,

concludes that sustaining fish supplies from capture fisheries will not be able to meet the growing global demand for aquatic food and importance of aquaculture will further be increasing. Today, aquaculture is probably the fastest growing food-producing sector. During the last 50 years world-wide production by aquacultures has already increased from less than a million tonnes in the early 1950s to 59.4 million tonnes in 2004, and it is still growing. Thus, today's level of production has a value of more than 70 billion US $ and accounts for almost 50% of the world's food fish production. In 2004, 41.3 million tonnes accounting for 69.6% of the global aquaculture production was produced in China (Fig. 12.1). Together with

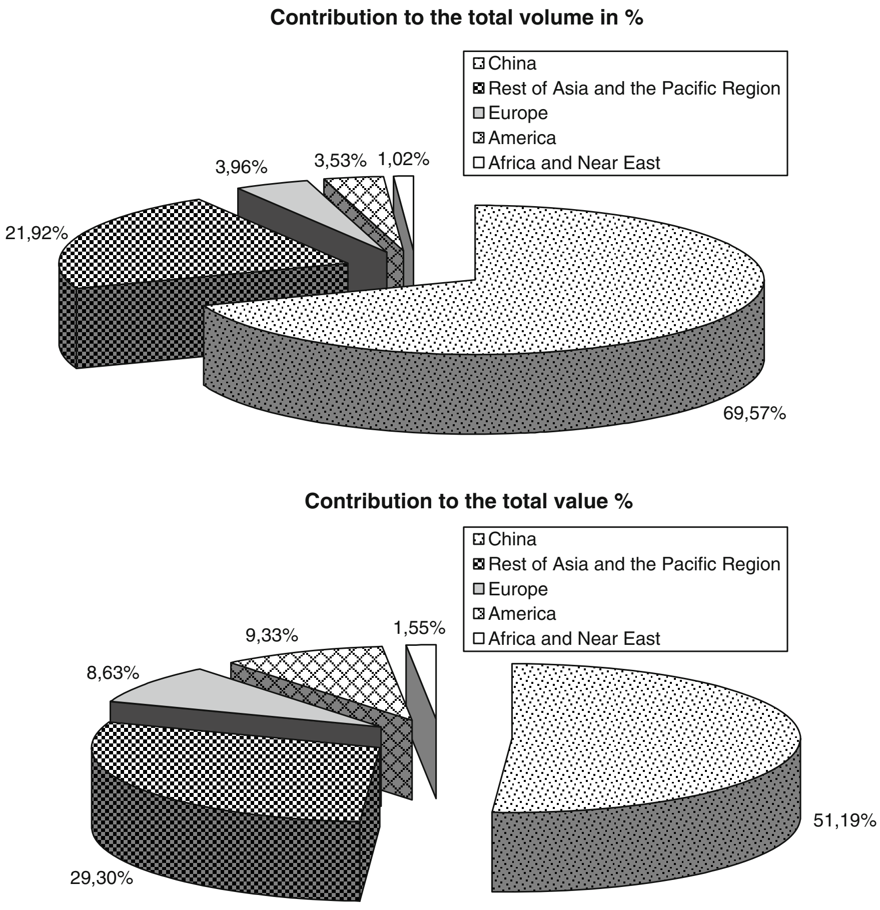


Fig. 12.1 Regional contributions (by volume and value) to the world aquaculture production in 2004. Data has been collected from the report of the Fisheries and Aquaculture Department of the FAO (FAO 2006)

the rest of Asia and the Pacific region production of food from aquacultures accounted for 91.5% of the total global production volume. Contributions from other regions were much lower by volume but contributed more significantly to cash value (Fig. 12.1). Thus, the 2.1 million tonnes of aquaculture products produced in 2004 in the Western European region contributed only 3.5% of the total volume but was valued at 5.4 billion US$ corresponding to a share of 7.7% of the total value. In 2004, aquaculture production from mariculture was estimated at 30.2 million tonnes, representing 50.9% of the total global production, whereas freshwater aquaculture contributed 25.8 million tonnes, or 43.4%. The remaining 5.7%, or 3.4 million tonnes, came from production in brackish environments (FAO 2006).

On the basis of the population growth projected for the next two decades, it is estimated that at least an additional 40 million tonnes of aquatic food will be required by 2030 to maintain the current per capita consumption (FAO 2006). This demand will only be satisfied by additional production from aquaculture. Intensive cultivation of animals within a limited environment may inevitably result in negative interactions. Thus, the Fisheries and Aquaculture Department of the FAO also mentions several cases of environmental and natural resources interactions that have been negatively associated with aquaculture (FAO 2006):

1. Discharge of aquaculture effluent leading to degraded water quality (eutrophication, concern over red tides, low dissolved oxygen, etc.) and organic matter rich sediment accumulation in farming areas
2. Alteration or destruction of natural habitats and the related ecological consequences of conversion and changes in ecosystem functions
3. Competition for the use of freshwater
4. Competing demands with the livestock sector for the use of fish meal and fish oil for aquaculture diets
5. Improper use of chemicals raising health and environmental concerns
6. Introduction and transmission of aquatic animal diseases through poorly regulated translocations
7. Impacts on wild fisheries resources through collection of wild seed and brood animals
8. Effects on wildlife through methods used to control predation of cultured fish

This chapter especially addresses issue number 5 from this list but also touches some of the other topics. The problem with modern fish farming is the high density of fish and the monoculture nature of aquaculture that allows the spread of diseases and parasites on a scale not previously seen in nature. This requires the use of pharmaceuticals on a large scale. Residues of antibiotics applied as veterinary drugs in aquaculture enter the water-cycle and easily settle into the sediments by direct exposure, via fish feed, or via fish excrements (Halling-Sørensen et al. 1998; Richardson and Bowron 1985). In this particular mode of application, substantial amounts of the drugs are neither metabolized nor degraded (Rigos et al. 2004; Hamscher 2006). Thus, much of the active agent reaches the benthic zone (the sea or lake bottom sediments) where it can accumulate in various organisms such as oysters (Kennedy et al. 2000).

12.2 Agents Used for Sex Reversal in Fish

12.2.1 Introduction

Pharmaceutical agents are routinely used to create monosex cultures of fish. This may be done to prevent loss of metabolic energy to reproductive processes, to take advantage of the higher weights obtained in one of the sexes or to take advantage of greater resistance to disease. They are also used to create sterile triploid fish for stocking streams and lakes for sport fishing. The most common species treated are tilapia, salmon, medaka, and Atlantic halibut (*Hippoglossus hippoglossus* L.). The most commonly used compounds are 17α-methyltestosterone (MT, Fig. 12.2) but 17α-methyldihydrotestosterone, norhisterone, trenbolone (Sect. 13.2.1 in Chap. 13) and nortestosterone are also used. Compounds such as dihydrotestosterone and methyldihydrotestosterone would be expected to be more effective compounds as the compounds are not aromatized to estrogen.

12.2.2 Methyltestosterone

The estimated amount of MT used for tilapia production in the US is about 1–2 kg which would represent a very small amount of the total MT produced per year (Green and Teichert Coddington 2000). Dissolved MT from feed in water and sediment has a half life of 1 week. The recommended levels of MT in the effluent from sex reversal units are 1 μg/L. The same phyla which are affected by steroid hormones would be expected to be influenced by MT, e.g. marine mollusks (LOEC 100 ng/L; Schulte-Oehlmann et al. 2004) or fish (Table 12.1); however, nematodes do not appear to be affected (Table 11.2).

12.2.3 Management

The amounts of steroids used can be reduced by using immersion shortly after hatching rather that adding large amount of active agents to the feed. Sex reversal can also be accomplished for tilapia by hybridization of species. It is also possible that phytoestrogens may be of use in some species (Dabrowski et al. 2004).

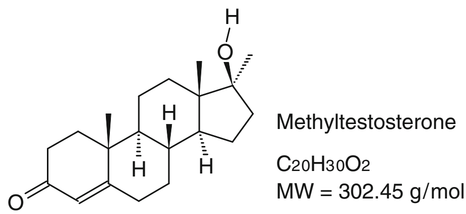


Fig. 12.2 Structural formula of the steroid hormone methyltestosterone

Table 12.1 Effects of methyltestosterone on fish at environmental relevant concentrations

Species			
Danio rerio	LOEC 26 ng/L	20-days post hatch Sex reversal, reduced vitellogen	Orn et al. (2003)
Oryzias latipes	LOEC 10 ng/L	Full life cycle Masculinization	Seki et al. (2004)
Fundulus heteroclitus	LOEC 1 ng/L LOEC 10 ng/L	14 days Decreased plasma T Decreased plasma E2	Sharpe et al. (2004)
Pimephales promelas	LOEC 200 ng/L	12 days Cessation of egg laying Secondary sexual characteristics	Ankley et al. (2001)

12.3 Use of Antibiotics in Aquaculture

12.3.1 Introduction

Some antibiotics administered to fight bacterial infections in human medicine are also used for the treatment of animals. After the discovery of the disease fighting but also growth promoting capabilities of antibiotics, livestock producers and fish farmers began using such drugs in animal feeds (Chap. 9). Today, treatment of animals is conducted either for therapy, for prophylactic (or metapyhlactic) reasons or just for growth promotion. For the last-named purpose, antibiotics are usually used in sub-therapeutic doses which have been recognized to contribute to promoting resistance. There is some scientific evidence that certain uses of antibiotics in food-producing animals can lead to antibiotic resistance in intestinal bacteria (Chap. 10). This resistance may then be transmitted to the general population causing treatment-resistant illness. Such uses of antibiotics can, however, also create antibiotic resistance in non-pathogenic bacteria. Resistance genes from non-pathogenic bacteria can be transferred to disease-causing bacteria that may cause antibiotic-resistant infections in humans (FAO 2005).

In Europe, only a limited number of pharmaceuticals are registered and may therefore be used in aquaculture produced animals or animal products for human consumption. But world-wide and especially in the Eastern Asian countries, which are the main producers of aquaculture products, a wide variety of animal drugs including more than 60 antibiotics are used (PAN 2003). In Japan, 26 different compounds were permitted to be used for treatment in aquaculture in the 1990s (Alderman and Hastings 1998). In 2000, Holmström et al. (2003) interviewed 76 Thai shrimp farmers concerning their usage of antibiotics in shrimp pond management. 74% of the interviewees confirmed the use of at least 13 different antibiotics. Holmström et al. (2003) ascertained that norfloxacin, oxytetracycline, enrofloxacin and sulfonamides were primarily used in “shrimp farming” in Thailand. In general, the extensive use of antibiotics in aquaculture is controversially debated especially with regard to antimicrobial resistance. In particular, the use of fluoroquinolone antibiotics as animal drugs is regarded as being very crucial because WHO listed these compounds as highly important antimicrobial drugs needed for human therapy.

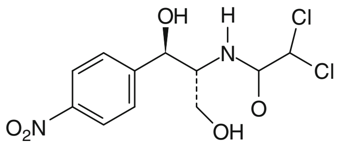
12.3.2 The Rapid Alert System for Food and Feed

The Rapid Alert System for Food and Feed (RASFF) is a system established in 1979 by the European Food Safety Authority (EFSA) on the legal basis of the EU Regulation EC/178/2002. Its purpose "is to provide the control authorities with an effective tool for exchange of information on measures taken to ensure food safety" (http://ec.europa.eu/food/food/rapidalert/index_en.htm). In 2007, 58 notifications have been by the RASFF for residues of veterinary medicinal products in fishery products (European Communities 2008). In the past 2 years, the number of RASFF notifications for drug residues in fishery products decreased from 104 (2005) to 80 (2006) and 58 (2007) (European Communities 2007, 2008).

Most of the notifications (35 in 2007 compared to 57 in 2006) concerned findings of nitrofurans, primarily as their metabolites. Most of these findings were residues in shrimp from India, China, Bangladesh and Vietnam but also a few other countries. A steep decline of the number of notifications for residues of chloramphenicol was observed between 2002 (113) and 2005 (2). Nevertheless, there is still some evidence of its illegal use proven by a stagnating number of notifications in 2006 and 2007 (5 each year). Other positive findings of residues resulting in RASFF notifications in 2007 have been issued for enro-/ciprofloxacin in fish and oxytetracycline in fish and crustaceans. Apart from residues of antibiotics two other compounds, malachite green and crystal violet and their respective metabolites (see Sect. 12.4), are also frequently found in fishery products and have been given notification by RASFF (50, 17 and 9 for malachite green and 2, 5 and 2 for crystal violet in 2005, 2006 and 2007, respectively) (European Communities 2007, 2008).

12.3.3 The Case of Chloramphenicol

In 2001, the detection of chloramphenicol (Fig. 12.3) residues in imported giant prawns from Asia triggered a discussion within the EU about how to deal with foods containing residues of pharmacologically active substances whose use is prohibited in food-producing animals in the EU. Chloramphenicol is listed in Annex IV (prohibited substances) of Council Regulation No. 2377/90/EEC as it may cause adverse human health effects (potential induction of aplastic anemia and positive results in genotoxicity testing). Zero tolerance applies to residues of such compounds but also to residues of any other non-registered pharmaceutically



Chloramphenicol

$C_{11}H_{12}Cl_2N_2O_5$
MW = 323.13 g/mol

Fig. 12.3 Structural formula of the "illegal" antibiotic drug chloramphenicol

active compound (all compounds not listed in Annexes I-III of Council Regulation No. 2377/90/EEC) occurring in food of animal origin intended for human consumption. This means that such food is not marketable if any of these compounds is detected at any concentration because risk cannot be excluded with regard to the precautionary principle.

The case of chloramphenicol also sparked great controversy because the detection of only low levels of chloramphenicol residues of between 0.1 and 1.0 μg/kg resulted in the destruction of tonnes of shrimps. It was also claimed that these residues may derive from unavoidable environmental background contamination, a hypothesis that has never been proven for chloramphenicol but has been demonstrated for other compounds (Kennedy et al. 2000). However, this case also resulted in the establishment of so-called minimum required performance limits (MRPLs) for certain substances which must be met by all official EU laboratories (e.g. 0.3 μg/kg for residues of chloramphenicol). MRPLs are based purely on analytical data and may be corrected downwards at any time and any other higher performance methods available and validated may also be used. MRPLs have in the meantime only been established for a few compounds (currently for ten substances/groups of substances). From a legal point of view, these values are not sufficient for verifying zero tolerances. But "as not to impede international trade whilst, at the same time, upholding the zero tolerance principle, the assessment of the importability of foods with residues of pharmacologically active substances was regulated with the help of decision 2005/34/EC. It specifies the use of the MRPL values of criteria decision 2002/657/EC as reference points for action. Hence foods with residue concentrations, which correspond to or exceed the value of the corresponding MRPLs, are to be intercepted at the time of import according to the current draft decision. The consignments are to be destroyed or sent back to their countries of origin." It also needs to be emphasized that technical feasibility alone and not the health risk was and still is the yardstick for the establishment of a MRPL value for a respective substance and that MRPLs have not normally undergone any risk assessment! (Heberer et al. 2007).

12.3.4 Avermectins

Avermectins like ivermectin (Fig. 12.4) and emamectin are used to control sea lice (*Caligus elongates*; *Lepeophtheirus salmonis*) particularly in salmanoid species.

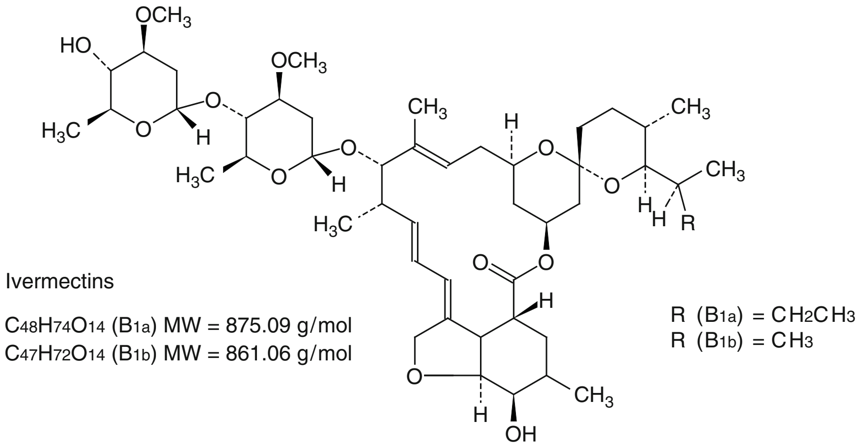


Fig. 12.4 Structural formulae of the antibiotic drugs ivermectin B_{1a} and B_{1b}

However, ecological concerns are also warranted as disturbing the bioturbating (generally the polychaetes or marine worms which create tunnels in the sediment) fauna living in the sediment close to fish farms which are vital in stimulating the rate of microbial degradation and the remineralisation of waste products. Without these processes the sediment quickly becomes anoxic. The resulting increase in hydrogen sulphide levels in the water column may, in turn, have a detrimental effect on fish health. The increased nutrient mass under and near fish farms, encourages the capitellid polychaetes. Ivermectin at relevant doses in the sediment was lethal to some of the species and in general caused a loss of biomass of oligochaeta (Collier and Pinn 1998). The most sensitive representative benthic species investigated to date is the marine annelid *Arenicola marina* which had a 10-day LC_{50} value of 23 μg/kg (Davies et al. 1998). Ivermectin is toxic to the two species used for environmental effects on sediments, *Corophium volutator* (shrimp) and *A. marina,* and sublethal effects have been observed for *A. marina* (Allen et al. 2007).

12.3.5 Oxytetracycline and Other Antibiotics

Samuelsen et al. (1992) detected oxytetracycline (Fig. 12.3), a veterinary drug frequently used in aquaculture, at concentrations up to 285 mg/kg in sediments collected from a fish farm. The same compound has also been detected at lower concentrations in marine sediments (Jacobsen and Berglind 1988; Capone et al. 1996; Lalumera et al. 2004) which demonstrates the widespread occurrence and exposure of aquatic sediments to oxytetracycline, most likely originating from aquaculture. Le and Munekage (2004) and Le et al. (2005) detected trimethoprim, sulfonamides, oxolinic acid and norfloxacin in water and sediment samples from aquaculture in

Vietnam. They detected sulfamethoxazole, trimethoprim, norfloxacin and oxolinic acid with maximum concentrations 2.39, 1.04, 6.06, and 2.50 mg/kg in water samples and 820, 735, 2,616, and 426 mg/kg wet weight in mud, respectively, collected from shrimp ponds in mangrove areas in North and South Vietnam.

12.3.6 Risk Characterization/Antibiotic Resistance

Direct exposure to antibiotics used in aquaculture are not generally considered as an environmental threat because such residues are diluted and by their nature do not directly affect benthic species. Ciprofloxacin, enrofloxacin, erythromycin, oxolinic acid and flumequine are not considered harmful to aquatic species. Similarly, the herbicides atrazine, simazine and terbutryn were not toxic but glyphosate was considered harmful by EU classification (Hernando et al. 2007). In contrast, Cabello (2006) points out that the often prophylactic use of antimicrobials in aquacultures may result both in environmental or human health risks. Potential risks are the direct or indirect transfer of resistant bacteria, the development of reservoirs of transferable resistance genes in bacteria in aquatic environments, and antimicrobial residues in edible fish, fish products and in the environment (Cabello 2006; FAO 2006; WHO 2006).

Kerry et al. (1995) identified fish feed as a source of oxytetracycline-resistant bacteria in the sediments under a marine fish farm. In their experiment, 529 kg of oxytetracycline were administered over a 24-day period with an average dose of 1.4 kg/cage/day. Three days after finishing the therapeutic application, Kerry et al. (1995) measured an average of 4.6 mg/kg of oxytetracycline residues in the sediments underneath the fish farm with an average frequency of antibiotic resistance of 9% in the microflora. This frequency of resistance increased to 26% twenty-four days after the end of the treatment. An even higher frequency of resistance of 36% was measured in the microflora isolated from mussels suspended above the sediments. Lalumera et al. (2004) identified oxytetracycline and flumequine as potential priority chemicals to be monitored for possible environmental side effects of aquaculture in Italy. They measured maximum residues levels up to 246 and 579 μg/kg dry weights for oxytetracycline and flumequine, respectively, in sediment samples collected from areas and surroundings of two trout farms and three sea-bass farms. In toxicity testing using a bioluminescence assay EC_{50} values varied between 12–15 mg/L for flumequine and 121–139 mg/L for oxytetracycline. In a study with fish reared in a prototype marine integrated system, Giraud et al. (2006) observed a selection towards oxolinic acid resistance in the intestines of fish treated with this quinolone antibiotic. Ho et al. (2000) tested the antibacterial activities of several antibiotics against aquatic bacterial isolates from soft-shell turtles, fish and shell fish raised in aquacultures in Taiwan. Antibiotic-resistant strains were observed for amoxicillin and oxytetracycline in more than 80% of the soft-shell turtle isolates ($N=98$), in 56% (amoxicillin) or 81% (oxytetracycline) of the fish isolates ($N=110$) and with around 50% in shellfish isolates ($N=46$). In total, 38, 17, 65, 64, 9, and 78% of the

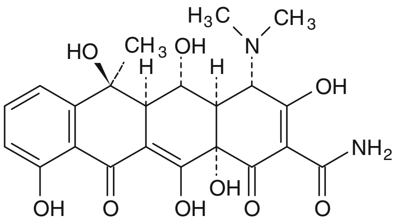


Fig. 12.5 Structural formula of the antibiotic drug oxytetracycline

254 investigated isolates contained drug-resistant strains against chloramphenicol, florfenicol, thiamphenicol, amoxicillin, oxolinic acid, and oxytetracycline, respectively. Samuelsen et al. (1992) found about 100% oxytetracycline-resistant bacteria in sediments of a fish farm at the end of a 10 days treatment with oxytetracycline (Fig. 12.5). Seventy two days later this rate dropped to 20% and stabilized at levels between 10 and 50%. Measured half-lives of oxytetracycline residues varied between 87 and 144 days.

Frequent antibiotic resistance was also found in investigations of shrimp aquacultures in Vietnam where *Bacillus* and *Vibrio* species were identified as being predominant among the resistant bacteria (Le et al. 2005). The incidence of resistance to sulfamethoxazole and trimethoprim was found to be significantly higher than to norfloxacin or oxolinic acid. Le et al. (2005) identified these four antibacterials as being environmentally relevant based on investigations of water and mud samples collected from shrimp ponds in mangrove areas in North and South Viet Nam (Sect. 12.3.5). Resistant bacteria were also found in investigations of costal fish from the Concepción Bay in Chile (Miranda and Zemelman 2001). High frequencies of resistance were observed to ampicillin, streptomycin and tetracycline, whereas resistance to chloramphenicol was rather low. Isolates from demersal fish (bottom feeders) generally exhibited resistance to more compounds (8–10) than those of from pelagic (non-bottom feeders) fish (7 and less). Miranda and Zemelman (2001) regarded the results as a direct link between disposals of urban sewage and the occurrence of antibiotic resistant bacteria in Chilean commercial fish residing in the receiving costal waters and prompting a potential risk to public health of fish consumers (Miranda and Zemelman 2001).

Penders and Stobberingh (2008) investigated the prevalence and degree of antibiotic resistance in Dutch catfish and eel farms using motile aeromonads as indicator bacteria. They found a prevalence of resistance of 100, 100, 24, 3, 3, and 0% for ampicillin, oxytetracycline, sulfamethoxazole, trimethoprim, ciprofloxacin, and chloramphenicol, respectively. The authors concluded that these fish farms are a major reservoir of oxytetracycline resistance genes that might also form a risk for human health.

In 2006, a joint FAO/OIE/WHO expert consultation on "antimicrobial use in aquaculture and antimicrobial resistance" identified and rated risks for public health in relation to the use of antimicrobials in aquaculture (WHO 2006). They concluded that “the issue of direct transmission of resistant human pathogenic bacteria represents a low risk as such bacteria are rarely transmitted through aquaculture products. The greatest risk to public health associated with antimicrobial use in aquaculture is assumed to be the development of a reservoir of transferable resistance genes in bacteria in aquatic environments. Such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens, and thereby potentially cause treatment problems due to resistance. Antimicrobial residues represent a low, but still significant public health risk” (WHO 2006). The report from the invitational European Union conference on The Microbial Threat (EU 1998) recognizes the transmission of resistant micro-organisms from animals to humans through the food chain as the major route which is also confirmed by other authors (Nawaz et al. 2001). Consumption of adequately cooked food and good kitchen hygiene reduces human health risks by resistant and/or pathogen bacteria to a minimum. Special food habits such as the consumption of raw fish (Sashimi or Sushi) also getting more and more popular in Western countries may however also be a source for the ingestion of such bacteria. In general, knowledge on impacts and sustainability of the extended use of antimicrobials in aquacultures on the aquatic environment is still very limited and conclusions will be speculative.

12.3.7 Risk Management

The use of antibacterial agents in aquaculture is a controversial issue. Especially the creation, transmission (bacteria or genes) or promotion of antibiotic-resistant bacteria is regarded as a potential and highly important threat to human health care. Today, many antibacterial drugs are banned for the use in aquaculture. Nevertheless in face of the growing demand for aquacultural products, further efforts for the substitution of antibacterial drugs in fish farming such as alternative health management procedures are necessary. Antibiotics should always be used responsibly and under adequate control through appropriate regulation. The following responsibilities have therefore been outlined by the FAO (2006) as being important to control the use of antibiotics and other veterinary drugs in fish farming:

- Awareness building and education of farmers and processors on the responsible use of therapeutics
- Pharmaceutical manufacturers and dealers, feed manufacturers, and other relevant service providers should also fully cooperate in the efforts to regulate therapeutic use in aquaculture
- Introduction of changes or tightening of national regulations on the use of therapeutics in general, and within the aquaculture sector in particular
- Stringent requirements for export trade

Excellent experiences were obtained using “cluster management” concepts which bring small-scale shrimp farmers together to manage their ponds jointly with better management practices (FAO 2006). Such concepts can either reduce the total amounts of antibacterials to be used and may also prevent the individual farmer from using banned veterinary medicinal products.

Another possible solution is the development of effective vaccines which may significantly reduce the use of antibiotics and can also increase production volumes. In Norway, a sharp decline in the use of antibiotics in fish (salmon) farming was observed after the development and application of a vaccine against furunculosis caused by the bacteria *Aeromonas salmonicida* (Midtlyng 2000). Despite a wide-spread abandonment of the use of antibiotics in fish farming since 1993, fish production in Norway increased more than five times between 1992 and 2003(FAO 2006). In Europe and North America, the use of antimicrobials is for most salmonid bacterial diseases and limited to emergency uses in the event of breakdown of vaccine protection (Alderman and Hastings 1998).

12.4 Triphenylmethane Dyes

12.4.1 Introduction

The triphenylmethane dyes malachite green (MG) (4-[(4-dimethylaminophenyl)-phenyl-methyl]-*N*,*N*-dimethyl-aniline) and crystal violet (CV) (4-[4,4-bis(dimethylamino) benzhydrylidene]cyclohexa-2,5-dien-1-yl-idene-dimethylammoniumchloride) (Fig. 12.6) are frequently used to treat fish or fish eggs infected by fungus *Saprolegnia* (Olah and Farkas 1978; Srivastava and Srivastava 1978) or by protozoa species such as *Ichthyophthirius multifiliis* (Schachte 1974) which parasitize freshwater fish causing “Ich” or the “white spot disease”, diseases that lead to major animal health problems for aquarists and commercial fish producers worldwide (Francis-Floyd and Reed 2002). Thus, MG became and still is very popular among fish farmers because of its broad fungicidal and anti-parasitical properties. MG and CV are readily absorbed from water into fish and reduced rapidly into their reduced colorless metabolites (Fig. 12.6) leucomalachite green (LMG) and leucocrystal violet (LCV) stored in the fatty muscle tissues (Bauer et al. 1988; Plakas et al. 1996; Thompson et al. 1999; Culp et al. 2006).

Neither MG nor CV is currently registered for use with food producing animals worldwide. MG was banned in 1983 in the US in food-related applications. In the EU, zero tolerance applies to all residues of MG or CV including their metabolites leucomalachite green (LMG) and leucocrystal violet (LCV) in foodstuffs because they are not listed in Annex I to III (registered substances) of Council Regulation No. 2377/90/EEC. In the EU, a MRPL of 2 μg/kg has been set for residues of MG as action limit for internationally traded food consignments (Commission Decision 2003). But despite all prohibitions for the use of MG or CV with food producing animals, consumers are exposed to such residues as demonstrated by frequent findings in fish and fish products, most likely resulting from illegal uses (see Sect. 12.3.2).

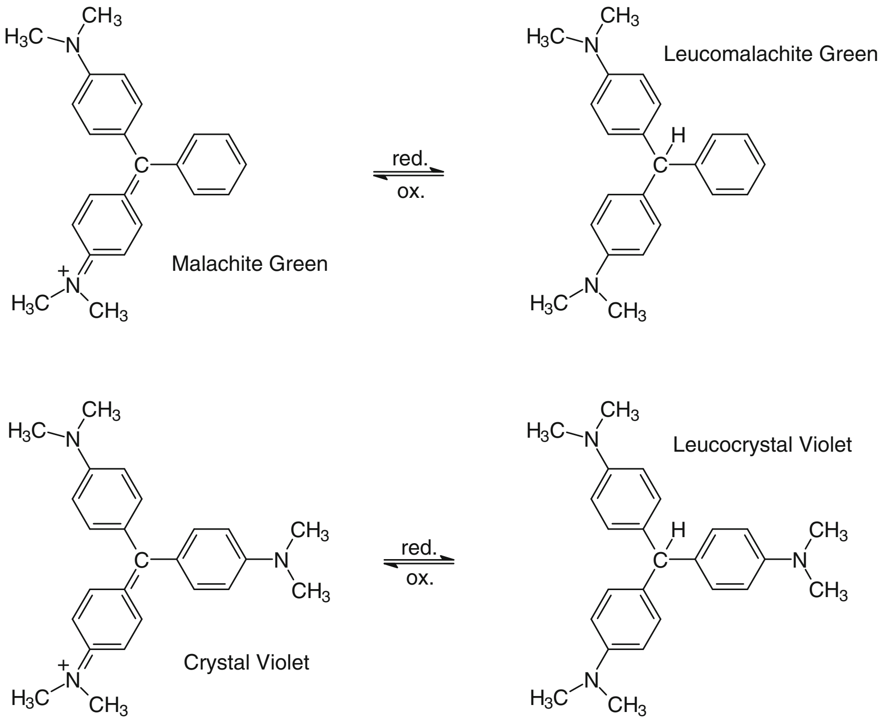


Fig. 12.6 Structural formulae of the triphenylmethane dyes malachite green and crystal violet (*left*) and their corresponding leuco metabolites (*right*)

Another issue which has only recently been addressed in investigations by Schuetze et al. (2008a, b) is the environmental occurrence of triphenylmethane residues originating from legal uses of MG and CV. Thus, both triphenylmethane dyes are legally used as veterinary drugs for the antiparasitic and/or antifungal treatment of ornamental fish. MG, CV and other methyl violet derivatives were or still are also used as pH indicator compounds, biological stains, gain medium, to detect latent blood in forensic medicine, hair dyes and for the coloring of textiles (e.g. with hypercolor clothes), paints, carbonless copy papers, ribbon tapes, printing inks and in medicinal products for human use.

12.4.2 Residues in Commercial Fish and Fish Products

In 2005, results from routine monitoring investigations at the Institute of Ichthyology in Cuxhaven (IFF Cuxhaven) yielded 14 positive detects out of 166 investigated fish tissue samples. Maximum residues were found in caviar of trout's from Sweden (619 μg/kg) (LAVES 2005a) and in an eel sample from China (3,911 μg/kg) (LAVES

2005b). Also in 2005, investigations by the Hong Kong Health Department showed that some samples of Chinese freshwater fish, crabs and other aquaculture products contained residues from the use of MG (Zhang 2005). This observation was also confirmed by Hong Kong's Food & Environmental Hygiene Department which detected MG residues up to 4,500 μg/kg (up to 900 μg/kg for fresh water fish) in 11 out of 14 eel-based products from local supermarkets. MG residues were also found in eight types of fresh-water fish from China including grass carp, mandarin carp, milk fish, snakehead fish and California perch (Zhang 2005). In 2007, residues of LCV were detected by the IFF Cuxhaven in samples of trout from two aquaculture farms in Lower Saxony, Germany. LMG was found with concentrations of 10 μg/kg and 35 μg/kg, respectively. The detection of LMG was regarded as proof of illegal use of MG for the production of food for human consumption. Both farms were shut-down immediately. Then follow-up samples were taken and investigated. Operation and sales were not allowed before zero tolerance for MG residues has been proven and could be guaranteed again (LAVES 2008).

12.4.3 Residues Detected in Environmental Samples

In a pilot study conducted in the city of Berlin, Germany, residues of MG, LMG, CV, and LCV have been detected in eels living in waters influenced by discharges of treated municipal sewage effluents (Schuetze et al. 2008a, b). Residues of MG and CV and especially of their corresponding leuco metabolites, LMG and LCV, were found in wild eels caught from surface waters downstream from the sewers of different municipal STPs. MG and LMG were detected with total concentrations up to 0.765 μg/kg fresh weight in the tissues of 25 out of 45 eels whereas CV and LCV where found with total concentrations up to 6.7 μg/kg fresh weight in 35 of these samples. The investigated samples were caught from different lakes, a river and a canal. In all cases, the occurrence of the residues in the samples could directly be linked to the presence of discharges by the municipal STPs into the receiving surface waters. The highest residues were found in those waters with the highest loads and portions of treated municipal sewage whereas no residues were found in an upstream lake which was not influenced by discharges from municipal sewers (Schuetze et al. 2008a, b). It should be pointed out that none of the investigated samples exceeded the MRPL value of 2 μg/kg (for the sum of MG and LMG) set as the action limit for internationally traded food consignments by the EU. This also means that these results obtained for a worst-case scenario may in return not explain any exceedance of this MRPL in (imported) fish by background contaminations originating from purified municipal sewage effluents!

MG and CV are multiple-use compounds also used to color materials. Thus, it appears reasonable that the residues of MG and CV found in the eel samples originated from such uses, e.g. from paints, printing inks or by wash off from clothes or paper towels colored with MG or CV. Additional loads from legal uses of MG or CV as veterinary drug for the treatment of ornamental fish (private aquaria) are also reasonable.

The results reported by Schuetze et al. (2008a, b) are the first proof of background contaminations of veterinary drugs found in samples of fish not intentionally treated with such agents. The findings also support some assumptions compiled in a recent review on MG by Sudova et al. (2007) who pointed out the persistence of MG residues in the environment and supposed that MG may turn up into untreated fish intended for human consumption. Sudova et al. (2007) warned that "every care must be taken when malachite green used for baths of aquarium or ornamental fish is being disposed of" and pointed out that "if enough attention is not paid to the problem, malachite green contained in baths or industrial waste water might penetrate to the aquatic environment and cause serious problems there". Thus, the hypothesis that MG residues may also turn up into untreated fish may now be considered to be substantiated.

12.4.4 Risk Assessment of MG Residues in Edible Fish

MG and LMG are regarded as potential genotoxic carcinogens (EFSA 2005a) making it impossible to derive and establish a tolerable daily intake (TDI). However, in line with the opinion of the European Food Safety Authority (EFSA 2005b), a "margin of exposure" (MOE) approach may be applied as a management tool to substances which are both genotoxic and carcinogenic to evaluate potential health risks for consumers. A MOE is, however, no substitute for a regular risk assessment based on an acceptable daily intake or a TDI and it still requires some dose–response related toxicological data. For MG and LMG current toxicological data are sufficient to conduct a "case-related" risk assessment on the basis of a MOE approach to evaluate potential consumer risks by contaminated foodstuffs (Heberer and Batt 2007). For the calculation of a MOE the following data are needed (1) the residue level in the individual food consignment (cs), (2) consumption data (cd) to calculate the Human Exposure (HExp = cd × cs) and (3) toxicological dose–response data to derive the lowest observed effect level*** (LOEL) → MOE = LOEL/HExp. With regard to protection of consumer health a margin of exposure below 10,000 is not regarded as being acceptable for genotoxic carcinogens (EFSA 2005b).

Table 12.2 shows the results for the calculation of the MOE for MG residues using different models for acute and chronic consumption. In this case the intake calculations were based on individual consumption data and the maximum residue found in eel samples (= 4,500 μg/kg) as reported by Zhang (2005). For all scenarios the MOE was clearly below 10,000. Especially the relevant and most realistic scenario based on acute consumption data for children revealed a MOE value of only 300 representing an unacceptable risk for human consumer's health.

Schuetze et al. (2008a) also applied the MOE approach to evaluate the human health risk associated with the consumption of eels contaminated by effluents of treated sewage at the highest residue found in their study. With a MOE of at least 1.8 million the risk was classified as being very low and the MG and LMG residues detected as environmental background contamination in the eel samples did not give

Table 12.2 Calculation of the "margin of exposure" (MOE) using different models for acute and chronic consumption

Consumption model/data	Calculated intake(μg/kg bw per day)	LOEL (mg/kg bw)	MOE
VELS: acute consumption data[a] (child 2–5 years, 16.15 kg bw, 152.5 g fish per day)	42.49	13	306
VELS: chronic consumption data[a] (child 2–5 years, 16.15 kg bw, 5.6 g fish per day)	1.56	13	8,331
CVMP (EMEA): chronic consumption data[b] (adult 60 kg bw, 300 g fish per day)	22.5	13	577

Intake calculations are based on individual consumption data and the maximum residue found in eel [= 4,500 μg/kg as reported by Zhang (2005)]. *bw* Body weight, *LOEL* lowest observed effect level obtained from the studies of the US National Toxicology Program (2005)

[a] Consumption data obtained from the VELS consumption study for children aged between 2 and 5 years (Banasiak et al. 2005)

[b] Consumption data from the risk analysis approach for residues of veterinary medicinal products in food of animal origin used by the Committee for Medicinal Products for Veterinary Use (2001) of the European Medicines Agency (EMEA)

any significant concerns with regard to potential adverse health effects for a single or for casual consumption. Nevertheless, Schuetze et al. (2008a) concluded that "due to their potential to act as genotoxic carcinogens, any oral exposure to residues of MG and LMG should be avoided". In the EU, zero tolerance generally applies to all residues of MG and LMG found in food for human consumption because MG is not registered for use as a veterinary drug for food-producing animals.

12.4.5 Potential Environmental Impacts

Even after destruction of MG by photolysis, compounds very toxic to aquatic life still remain (most prominently, 4-(dimethylamine)benzophenone; EC_{50}, 30 min, 61 ng/L) in the *Vibrio fischeri* (a bacteria) test (Pérez-Estrada et al. 2008). MG itself is considered toxic to aquatic organisms (EC_{50}, 30 min, 31 ng/L) (Hernando et al. 2007).

12.4.6 Management Options

Neither MG nor CV is allowed to be used for the treatment of food-producing animals. Thus, efficient food control and management systems will limit illegal uses in commercial fish production to a minimum. However, other legal uses such as applications to ornamental fish will not be affected. To date, there is also no information on the occurrence of triphenylmethane dye residues in the effluent from ornamental fish CAFOs. Considering the apparently widespread exposure of non-target organisms to

MG, CV and their metabolites, a full investigation of ornamental fish CAFO effluent appears to be merited. Sudova et al. (2007) discussed potential alternatives for the use of MG in aquacultures in a recent review article. They also emphasized the difficulties in substituting MG for the treatment of fish eggs and fish and concluded that "in spite of partial success in the treatment of some diseases using…'replacement' preparations, no truly adequate substitute for malachite green has been found". Alternatives may, however, be available for the multiple uses of MG or CV, e.g. for the coloring of textiles, as such dyes are outdated.

References

Alderman DJ, Hastings TS (1998) Antibiotic use in aquaculture. development of antibiotic resistance – potential for consumer health risks. Int J Food Sci Technol 33:139–155

Allen YT, Thain JE, Haworth S, Barry J (2007) Development and application of long-term sublethal whole sediment tests with *Arenicola marina* and *Corophium volutator* using Ivermectin as the test compound, Environ Pollut 146:92–99

Ankley GT, Jensen KM, Kahl MD, Korte JJ, Makynen EA (2001) Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 20:1276–1290

Banasiak U, Heseker H, Sieke C, Sommerfeld C, Vohmann C (2005) Abschätzung der Aufnahme von Pflanzenschutzmittel-Rückständen in der Nahrung mit neuen Verzehrsmengen für Kinder. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 48:84–98

Bauer K, Dangschat H, Knöppler H-O (1988) Aufnahme und Ausscheidung von Malachitgrün bei Regenbogenforellen. Arch Lebensmittelhyg 39:97–102

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8:1137–1144

Capone DG, Weston DP, Miller V, Shoemaker C (1996) Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture 145:55–75

Collier LM, Pinn EH (1998) An assessment of the acute impact of the sea lice treatment ivermectin on a benthic community. J Exp Mar Biol Ecol 230:131–147

Culp SJ, Mellick PW, Trotter RW, Greenlees K–J, Kodell RL, Beland FA (2006) Carcinogenicity of malachite green chloride and leucomalachite green in B6C3F1 mice and F344 rats. Food Chem Toxicol 44:1204–1212

Dabrowski K, Rodriguez G, Lee K-J, Abiado MAG, Sanchez WC, Marquez-Couturier G, Phelps R (2004) Studies on fate of methyltestosterone and its metabolites in tilapia and on the use of phytochemicals as an alternative method to produce a monosex population of tilapia. In: Harris R, Courter I, Egna H (eds) Twenty-first annual technical report. Aquaculture CRSP, Oregon State University, Corvallis, OR, pp 53–60

Davies IM, Gillibrand PA, McHenery JG, Rae GH (1998) Environmental risk of ivermectin to sediment dwelling organisms. Aquaculture 163:29–46

EFSA (European Food Safety Authority) (2005a) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) to review the toxicology of a number of dyes illegally present in food in the EU. EFSA J 263:1–71

EFSA (European Food Safety Authority) (2005b) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J 282:1–31

EU (European Union) (1998) Conference on the microbial threat. The Copenhagen recommendations. Report from the invitational EU conference on the microbial threat, Copenhagen, Denmark, 9–10 Sept 1998 as cited in FAO (2005)

European Communities (2007) The Rapid Alert System for Food and Feed (RASFF): Annual Report 2006, Office for Official Publications of the European Communities, Luxembourg, http://ec.europa.eu/food/food/rapidalert/index_en.htm. Accessed 04 Sept 2008

European Communities (2008) The Rapid Alert System for Food and Feed (RASFF): Annual Report 2007, Office for Official Publications of the European Communities, Luxembourg http://ec.europa.eu/food/food/rapidalert/index_en.htm. Accessed 04 Sept 2008

FAO (2005) Responsible use of antibiotics in aquaculture. FAO fisheries technical paper no. 469. Food and Agriculture Organization of the United Nations (FAO), Rome

FAO (2006) The state of world fisheries and aquaculture 2006. FAO fisheries and aquaculture department, FAO fisheries technical paper no. 500. Food and Agriculture Organization of the United Nations (FAO), Rome

Francis-Floyd R, Reed P (2002) *Ichthyophthirius multifiliis* (white spot) infections in fish. CIR920 of the Fisheries and Aquatic Sciences Department Series, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida 1991, revised July, 2002. http://edis.ifas.ufl.edu/pdffiles/FA/FA00600.pdf. Accessed 06 May 2008

Giraud E, Douet DG, Le Bris H, Bouju-Albert A, Donnay-Moreno C, Thorin C, Pouliquen H (2006) Survey of antibiotic resistance in an integrated marine aquaculture system under oxolinic acid treatment. FEMS Microbiol Ecol 55:439–448

Green BW, Teichert Coddington DR (2000) Human food safety and environmental assessment of the use of 17 alpha-methyltestosterone to produce male tilapia in the United States. J World Aquacult Soc 31:337–357

Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere 36:357–393

Hamscher G (2006) Veterinary pharmaceuticals. In: Reemtsma T, Jekel M (eds) Organic pollutants in the water cycle. Wiley-VCH, Weinheim, pp 99–120

Heberer T, Batt N (2007) Risk assessment of malachite green residues - literature study (prepared by Germany). Agenda Item 9, CRD 9, Joint FAO/WHO Food Standards Programme Codex Committee on Residues of Veterinary Drugs in Foods. 17th Session, Breckenridge, Colorado, USA

Heberer T, Lahrssen-Wiederholt M, Schafft H, Abraham K, Pzyrembel H, Henning KJ, Schauzu M, Braeuning J, Goetz M, Niemann L, Gundert-Remy U, Luch A, Appel B, Banasiak U, Böl GF, Lampen A, Wittkowski R, Hensel A (2007) Zero tolerances in food and animal feed – Are there any scientific alternatives? A European point of view on an international controversy. Toxicol Lett 175:118–135

Hernando MD, De Vettori S, Martínez Bueno MJ, Fernández-Alba AR (2007) Toxicity evaluation with *Vibrio fischeri* test of organic chemicals used in aquaculture. Chemosphere 68:724–730

Ho S-P, Hsu T-Y, Che M-H, Wang W-S (2000) Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. J Vet Med Sci 62:479–485

Holmström K, Gräslund S, Wahlström A, Poungshompoo S, Bengtsson B-E, Kautsky N (2003) Antibiotic use in shrimp farming and implications for environmental impacts and human health. Int J Food Sci Technol 38:255–266

Jacobsen P, Berglind L (1988) Persistence of oxytetracycline in sediments from fish farms. Aquaculture 70:365–370

Kennedy G, Cannavan A, McCracken RJ (2000) Regulatory problems caused by contamination, a frequently overlooked cause of veterinary drug residues. J Chromatogr A 882:37–52

Kerry J, Hiney M, Coyne R, NicGabhainn S, Gilroy D, Cazabon D, Smith P (1995) Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms. Aquaculture 131:101–113

Lalumera GM, Calamari D, Galli P, Castiglioni S, Crosa G, Fanelli R (2004) Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. Chemosphere 54:661–668

LAVES (2005a) Malachitgrün in Forellenkaviar nachgewiesen. Press release of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES). http://www.laves.niedersachsen.de/master/C10047751_L20_D0_I826_h1.html Accessed 04 Sept 2008

LAVES (2005b) Weihnachts“karpfen: aus niedersächsischen Aquakulturen auf den Festtags-Tisch (in German). Press release No. 74 of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES). http://www.laves.niedersachsen.de/master/C15337944_L20_D0_I826_h1.html. Accessed 04 Sept 2008

LAVES (2008) LAVES untersucht Fisch aus Aquakulturen auf Rückstände. Press release of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES). http://www.laves.niedersachsen.de/master/C47273831_N15600592_L20_D0_I826.html Accessed 04 Sept 2008

Le TX, Munekage Y (2004) Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. Mar Pollut Bull 49:922–929

Le TX, Munekage Y, Kato S (2005) Antibiotic resistance in bacteria from shrimp farming in mangrove areas. Sci Total Environ 349:95–105

Midtlyng PJ (2000) Vaccination in salmonid aquaculture: A review. In: Fingerman M, Nagabhushnam R (eds) Recent advances in marine biotechnology. vol. 5. Immunobiology and pathology. Science Publishers, Enfield, NH, pp 227–242

Miranda CD, Zemelman R (2001) Antibiotic resistant bacteria in fish from the Concepcion Bay, Chile. Mar Pollut Bull 42:1096–1102

Nawaz MS, Erickson BD, Khan AA, Khan SA, Pothulari JV, Rafii F, Sutherland JB, Wagner RD, Cerniglia CE (2001) Human health impact and regulatory issues involving antimicrobial resistance in the food animal production environment. Reg Res Perspect 1:1–10

Olah J, Farkas J (1978) Effect of temperature, pH, antibiotics, formalin and malachite green on the growth and survival of *Saprolegnia* and *Achlya* parasitic on fish. Aquaculture13: 273–288.

Orn S, Holbech H, Madsen TH, Norrgren L, Petersen GI (2003) Gonad development and vitellogenin production in zebrafish (*Danio rerio*) exposed to ethinylestradiol and methyltestosterone. Aquatic Toxicol 65:397–411

PAN (2003) Chemikalieneinsatz in der Aquakultur. Pestizid Aktions-Netzwerk e.V. (PAN Germany), GTZ (GmbH), pp 90

Penders J, Stobberingh EE (2008) Antibiotic resistance of motile aeromonads in indoor catfish and eel farms in the southern part of The Netherlands. Int J Antimicrobia Agents 31:261–265

Pérez-Estrada LA, Agüera A, Hernando MD, Malato S, Fernández-Alba AR (2008) Photodegradation of malachite green under natural sunlight irradiation: Kinetic and toxicity of the transformation products. Chemosphere 70:2068–2075

Plakas SM, El Said KR, Stehly R, Gingerich WH, Allen JL (1996) Uptake, tissue distribution, and metabolism of malachite green in the channel catfish (*Ictalurus punctatus*). Can J Fish Aquat Sci 53:1427–1433

Rigos G, Nengas I, Alexis M, Troisi GM (2004) Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. Aquatic Toxicol 69:281–288

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. J Pharm Pharmacol 37:1–12

Samuelsen OB, Torsvik V, Ervik A (1992) Long-range changes in oxytetracycline concentration and bacterial resistance toward oxytetracycline in a fish farm sediment after medication. Sci Total Environ 114:25–36

Schuetze A, Heberer T, Juergensen S (2008a) Occurrence of residues of the veterinary drug malachite green in eels caught downstream from municipal sewage treatment plants. Chemosphere 72:1664–1670

Schuetze A, Heberer T, Juergensen S (2008b) Occurrence of residues of the veterinary drug crystal (Gentian) violet in wildlife eels caught downstream from municipal sewage treatment plants. Environ Chem 5:194–199

Schulte-Oehlmann U, Oetken M, Bachmann J, Oehlmann J (2004) In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn, Springer-Heidelberg Berlin, pp 233–247

Seki M, Yokota H, Matsubara H, Maeda M, Tadokoro H, Kobayashi K (2004) Fish full life-cycle testing for androgen methyltestosterone on medaka (*Oryzias latipes*) Environ Toxicol Chem 23:774–781

Sharpe RL, MacLatchy DL, Courtenay SC, Van Der Kraak GJ (2004) Effects of a model androgen (methyl testosterone) and a model anti-androgen (cyproterone acetate) on reproductive endocrine endpoints in a short-term adult mummichog (*Fundulus heteroclitus*) bioassay. Aquat Toxicol 67:203–215

Schachte JH Jr (1974) A short term treatment of malachite green and formalin for the control of *Ichthyophthirius multifiliis* on channel catfish in holding tanks. Prog Fish-Cult 36:103–104

Srivastava GC, Srivastava RC (1978) A note on the potential applicability of malachite green oxalate in combating fish-mycoses. Mycopathologia 23:774–781

Sudova E, Machova J, Svobodova Z, Vesely T (2007) Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: A review. Vet Med-Czech 52:527–539

Thompson HC, Rushing LG, Gehring T, Lochmann R (1999) Persistence of Gentian violet and leucogentian violet in channel catfish (*Ictalurus punctatus*) muscle after water-borne exposure. J Chromatogr B 723:287–291

WHO (2006) Antimicrobial use in aquaculture and antimicrobial Resistance. Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance, Seoul, Republic of Korea, 13–16 June 2006, Geneva, pp 107

Zhang X (2005) Hong Kong people fear poisonous Chinese fish. The Epoch Times, http://en.epochtimes.com/news/5-8-31/31823.html, Accessed 06 May 2008

Chapter 13
Organic Compounds Used in Animal Husbandry

Laurence Shore and Robert Wershaw

Abstract Although in general, the same drugs are used by humans as for animal husbandry, some compounds are unique to CAFOs. In raising and fattening steers, anabolic steroids are widely used in the US. In the cattle industry, large use is made of the acaricides, avermectins, and the cypermethrins as well as juvenile growth hormone inhibitor for fly and tick control that could affect soil fauna in very small quantities as they reach the environment without any modification. In poultry, the organic arsenides have been widely used for decades to control coccidiosis and increase growth. The environmental fate of the arsenic excreted in the poultry feces is therefore been an area of concern.

13.1 Introduction

Some chemicals are of particular interest for animal husbandry CAFOs either because they are not used for other purposes or reach the environment in a more concentrated form than from other sources. The ionophore antibiotics used exclusively in animal husbandry have been discussed in Chap. 9. This chapter will consider (1) the anabolic agents used in cattle growth, trenbolone and melengestrol acetate; (2) agents used in fly control; (3) agents used in helminth and tick control and (4) organic arsenic compounds use for growth in poultry. The excretion of the pharmaceuticals of concern in manure is shown in Table 13.1. However, concentrations of compound or their metabolites are of less concern than their biocidal activity against non-target organisms which is the usual parameter measured (Wardhaugh 2005).

L. Shore (✉) and R. Wershaw
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute, POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_13,

Table 13.1 Drug residues in manure

Compound		Reference
Trenbolone	1,700 pg/g liquid manure 75 ng/g solid manure	Schiffer et al. (2001)
Melengestrol	8 ng/g solid manure	Schiffer et al. (2001)
Roxarsone	30–50 mg/kg Arsenic	Garbarino et al. (2003)
Ivermectin	2.6 mg/kg dry wt grain fed	Cook et al. (1995)
	1.2 mg/kg dry wt pasture fed	Cook et al. (1995)
Doramectin	0.72 mg/kg dry wt	Dadour et al. (2000)

13.2 Anabolic Pharmaceuticals

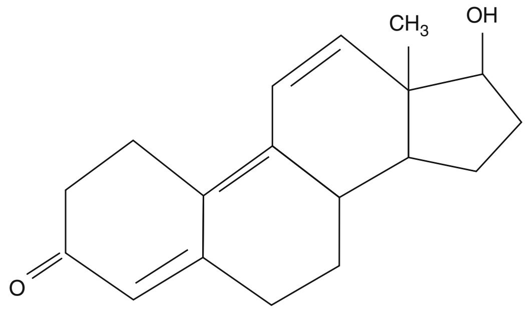
13.2.1 Trenbolone

The steroids trenbolone acetate (TbA) and melengestrol acetate (MGA) are licensed as growth promoters in the US but are not permitted in the EU and countries with similar regulations. TbA is released into the bovine blood stream, bile and feces as trenbolone-17α TbOH-17α; Fig. 13.1). In the heifer, only one major metabolic route occurs, oxidation of TbOH-17α to trendione, followed by reduction to TbOH-17β. Trenbolone had a half life of 275 days in liquid manure and was stable in solid manure for at least 4 months (Schiffer et al. 2001). After application, trenbolone was detectable in soil for 8 weeks. Similarly, MGA was stable in a manure pile for at least 4 months and was persistent in the soil for several months. In effluent from a cattle feed lot, TbOH-17α and TbOH-17β was found at maximum concentrations of 125 ng/l and 20 ng/l. Downstream (about 400 m) of the discharge, the concentrations were 50 and 5 ng/L respectively. However, a significant correlation between trenbolone concentrations and the androgenic activity (as measured using cells transfected with androgen receptor) of the river samples could not be demonstrated (Durhan et al. 2006). These levels are within the range shown in fish to cause masculinization of females and reducing fecundity (Table 13.1). In streams receiving effluent from feedlots in Nebraska, MGA (1–3 ng/l) was detectable while TbOH and zearalenol were not detectable (<1 ng/l) (Kolok et al. 2007).

13.2.2 Zearalenol

Other growth promoters, used alone or with trenbolone, are zearalenone and its more potent metabolite, α-zearalanol. These naturally occurring compounds are resorcylic acid lactones with estrogenic activity produced by *Fusarium* Spp. Its function in fungi is not known. Although many *Fusaria* found on various cereal (in particular corn or zea) crops (and possibly even in water supplies) produce zearalenone and zearalenol, soil *Fusarium* spp. apparently do not (Abbas et al. 1984;

Fig. 13.1 Trenbolone 17α



Russell and Paterson 2007). The amount of zearalenol present in the urine of animals treated with zearalanol is about the same found following ingestion of contaminated forage (Kennedy et al. 2000). The possible effects on soil and water ecology have not been examined. However, it can be argued that since the soil and water are frequently exposed to zearalenol with no noticeable effects and the soil bacteria rapidly degrade the compound (Mortensen et al. 2006), one would not expect the relatively small amount of the material excreted in the urine of feedlot steers to present a serious problem, especially since it has not been detected streams receiving cattle lot effluent (Kolok et al. 2007).

13.2.3 Clenbuterol

Clenbuterol [–(4-amino-3,5-dichloro-phenyl)-2-(tert-butylamino)ethanol] is a β_2 adrenergic agonist approved for use as a bronchodilator in horses. It is widely suspected to be used as an illegal growth promoter in domestic animals (Smith 2000). However, clenbuterol apparently is not persistent in the environment and has only rarely been reported in sewage treatment plant effluent or in receiving streams where dozens of other pharmaceuticals have been detected (Wilken et al. 2000; Rabiet et al. 2006).

13.3 Ecology of Cow Dung

The ecology of dung differs according to the amount of moisture and temperature but the main processes can be summarized as follows. Feeder flies lay their eggs in the newly formed dung. The resultant larvae attract predator wasps which kill the larva. The coprophagus beetles or earthworms create tunnels for microorganisms to multiply, which enhances degradation. The failure of the beetles to colonize the dung results in dung remaining on pasture and reduces the amount of nitrogen available to soil organisms.

The significance of dung beetles became apparent when cattle were introduced into Australia where the endogenous marsupial dung beetles could not degrade the dung. This resulted in proliferation of introduced and native flies and "pasture fouling" which obstructs plant growth and promotes rank unpalatable growth around the edge of dung pats (Tyndale-Bisoce 1996; Waterhouse and Sands 2001).

This presents two major problems for non-target species. (1) Within the CAFO, how does one kill the fly larva without destroying the predatory wasps and (2) how does one treat animals for mites and ticks on pasture without decreasing the population of coprophagus beetles.

Organic phosphates, such as chlorpyrifos and diazinon, and the pyrethrins (named after Pyrethrum flowers that produced a natural insecticide) are administered as ear tags, hanging strips or pour-ons for controlling ticks and flies so they do not reach the effluent. However some compounds are found in the feces for up to two weeks and need to be evaluated individually, both as to species (cows or sheep) and effects on non-target organisms (Wardhaugh 2005; Floate et al. 2005). Since the same compounds kill the predatory wasps, mites and beetles that destroy fly larva, it is counterproductive to use them in ways that reach the feces as the flies regenerate much more rapidly that their predators. Furthermore, flies very readily develop resistance to such agents. To kill the larvae in the feces, agents which selectively attack fly larvae, insect growth regulators (IGRs) are used. Alternatively, the parasitic wasps *(Muscidifurax raptor)* can be directly introduced to the feces.

13.3.1 Insect Growth Regulators

IGRs, principally methoprene, are juvenile hormone agonists that interfere with the larval development into adults. The IGRs are particularly effective against Dipterans and mosquitoes. Since methoprene is applied directly to the environment for mosquito control, its effects on many relevant environmental phyla have been assessed (Stark 2005). The main concerns have been on the effects on mutations in frogs and deleterious effects on dung beetles but neither of these effects has been confirmed in the general literature. Methoprene does not adversely affect pasture dung beetle populations in temperate climates (Bertone 2004). Methoprene has a very short lifetime under field conditions (2 days) but the bolus form given for cattle can be excreted for months in the feces. The effect on soil organisms has not been widely investigated as soil bacteria rapidly degrade the compound and it is not stable in sunlight.

13.3.2 Avermectins

The avermectins are used for control of helminths and other parasites. The avermectins are macrolides derived from soil yeast cultures and are of concern as they enter the environment unchanged, are used in relatively high quantities, and have a biocidal mode of action. Nearly all of the avermectins administered are excreted into cattle manure (98%) and can persist at sublethal doses up to 40 days after injection and when given as a bolus can last for a 100 days (Dadour et al. 2000). The avermectins in the feces reduced the population of dung beetles which are essential for dung digestion in dry climates (Dadour et al. 2000; Floate et al. 2005). However, in studies done in temperate climates neither the earthworms nor coleopterans were affected even though there was a decrease in dung decomposition (Madsen et al. 1990; Svendsen et al. 2002). The delay in dung decomposition was explained by the long term effect of the ivermectin on dipterans (Madsen et al. 1990). There is evidence that the effects of the avermectins in the field may be limited as there is re-colonization from unaffected cow dung pats. However, in the case where beetles or other insects are being introduced into a new habitat, the impact of biocidal drug residues may severely impede their habituation (Wardhaugh 2005).

13.4 Arsenic Organic Compounds

13.4.1 Introduction

Although technically arsenic organic compounds are antibiotics or at least limit bacterial activity, the environmental concern for the arsenic organic compounds used in poultry for coccidiosis control is the release of the inorganic arsenate [As(V)] and the more toxic arsenite [As(III)] into the environment rather than antibiotic resistance. The compounds are also used to improve feed efficiency and to promote rapid growth. The amount of As actually required for growth in poultry and swine is very small (>1 mg/kg) and the mechanism of action has not been defined (Nielsen 1998).

The most commonly used organic As is roxarsone (3-nitro-4-hydroxybenzene arsonic acid). It has been estimated that 10^6 kg/year (14–48 mg/kg litter) is released into the environment in the US (Garbarino et al. 2003). Organic arsenides have been banned in the EU since 1999 (Nachman et al. 2005).

Poultry litter composed of the manure and bedding material has a high nutrient content and is used routinely as a fertilizer on cropland and pasture. The toxic effects of inorganic As on ecosystems have been well documented in surface, soil and groundwater as naturally occurring inorganic As exists in many geographical areas (Environment Canada 1993). Therefore the As released from fertilization with poultry manure has been of concern, especially as it appears to be released in water soluble forms as described in the next section.

13.4.2 Degradation of Roxarsone During Composting

Roxarsone is stable in fresh dried poultry litter and the primary arsenic compound extracted with water is roxarsone (Garbarino et al. 2003). However, when water was added to litter at about 50 wt % and the mixture was allowed to compost at

40°C, the speciation of arsenic shifted from roxarsone to arsenate in about 30 days. Increasing the amount of water increased the rate of degradation. The rate of degradation was directly proportional to the incubation temperature and heat sterilization eliminated the degradation. That the degradation was primarily biotic was supported by results from enterobacteriaceae growth media that were inoculated with litter slurry to enhance the biotic processes and to reduce the concomitant abiotic effects from the complex litter solution. Similarly, Stolz et al. (2007) demonstrated that although most of the roxarsone is excreted as such, it is converted to inorganic As(V) by *Clostridium* sp. in poultry manure or slurry. Similar results have been observed for swine slurry (Makris et al. 2008).

13.4.3 Mobility of Arsenic in Soils Amended with Poultry Litter

When poultry litter is applied to agricultural fields, the arsenic is released to the environment and may result in increased arsenic in surface and groundwater and increased uptake by plants. In the Delmarva Peninsula (MD) total As was elevated in the soil following years of amendment with poultry litter (Gupta and Charles 1999). Elevation of trace metals like As in amended soil is not unusual as other trace elements like Cu, Zn and Mn, which are routinely added to poultry feed, are also elevated in amended soil (Gupta and Charles 1999; Han et al. 2000).

Of more importance than the total concentrations of trace metals is that these metals can readily enter the pore water of the soil. These water-soluble components are available for uptake by plants and other soil organisms. Elevated concentrations of metals in soil water can lead to elevated metal concentrations in plants, which may be phytotoxic or render the plants unsuitable for human or animal consumption (Krishnamurti and Naidu 2002; Marin et al. 1993). High metal concentrations in soil water can also contribute to surface water and groundwater contamination. In aerobic soils and sediments, As is strongly associated with clays and iron oxides (Grafe et al. 2002; Rutherford et al. 2003). The dissolution of oxides, when sediments become reducing (anaerobic; water logged) can cause a flux of As back to the water column. It therefore important to determine the distribution of water-soluble and more tightly bound fractions of As to understand the mobility of arsenic from agricultural operations and its potential to contaminate surface water and groundwater. Because collecting and measuring soil pore-water samples can be difficult and expensive, water extraction of the soils is used as being proportional to potential pore-water concentrations. It would be expected that water extraction would underestimate the actual pore-water concentrations (Blaser et al. 2000).

The release of arsenic from the poultry litter amended soils and unamended reference sites was examined by extraction with water and strong acids (HCl and HNO_3) following both short term (2 years) and long term amendment (Rutherford et al. 2003). The application of the poultry litter increased concentrations of water-extractable arsenic species compared to the reference sites. The hypothesis that water extractable arsenic is derived primarily from poultry litter amendments was supported by the following observations: (1) Mobilization of arsenic, as well as the

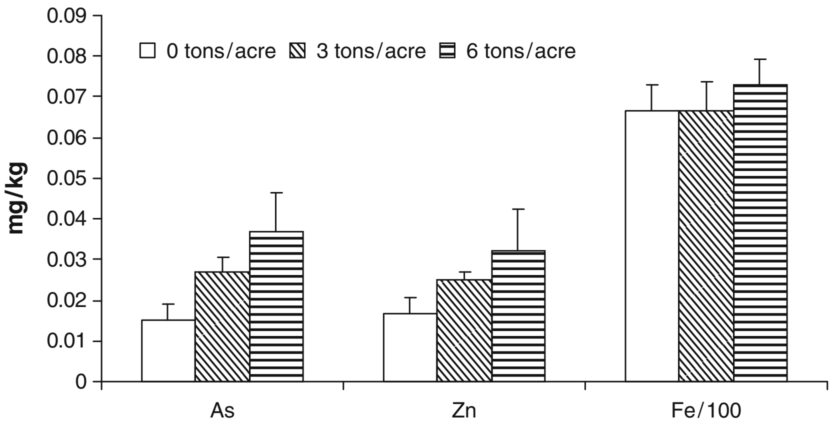


Fig. 13.2 Water extractable As (mg/kg), Zn (mg/kg) and Fe (100 mg/kg) in fields following no application or two applications of 3 or 6 tons of poultry manure/acre in the upper 15 cm of soil (data from Rutherford et al. 2003)

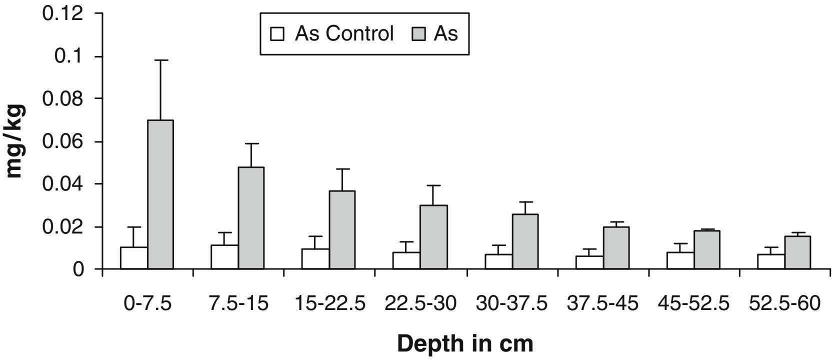


Fig. 13.3 Water extraction of soils from fields in with long term application of poultry litter compared with control site (based on Rutherford et al. 2003)

other feed additives, such as Cu and Zn, was observed when poultry litter was extracted with water. (2) There was a strong positive correlation ($r^2 > 0.90$) between water-extractable concentrations of As, C, P, Cu, and Zn in soils amended with poultry litter, and (3) these concentrations decreased with increasing depth (greater distance from the application of litter) (Fig. 13.2) and (4) the amount of arsenic added to a tilled portion of a field at a rate of 3 and 6 tons per acre increased the total arsenic concentration of a soil containing a background concentration of 3.5 mg/kg arsenic by 1.5–3% per application (Fig. 13.3).

Water extraction detected soluble As in the amended soil was elevated even though the total dissolution (water and acid extraction together) showed no difference in arsenic concentrations. This was observed in fields having only two applications of poultry litter as well as fields which had long-term application of litter.

Although the arsenic in the poultry litter is easily mobilized by water, its leach rate from amended soils appears to be slow enough that it accumulates in the soils. This was evidenced by a significant difference between water-extractable arsenic in the amended soil after one application at 6 tons per acre and after two applications at 3 and 6 tons per acre compared to the unamended plot. Even though these applications were 2 years apart, there was a measurable increase in water-extractable arsenic between the first and second applications.

The evidence suggests that, although arsenic oxides are usually strongly bound to the soil, the arsenides and arsenates in poultry manure amended fields tend to stay in soluble forms and thereby could be found in runoff and groundwater more readily than from natural arsenic sources. Furthermore, arsenic is not lost in incineration or biogas facilities nor is it acceptable for organic farming (Nachman et al. 2005). This potential environmental impact needs to be weighed against the advantages of using arsenides as growth promoters or coccidiostats (Jones 2007).

References

Abbas HK, Mirocha CJ, Shier WT (1984) Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. Appl Environ Microbiol 48:654–661

Bertone MA (2004) Dung beetles (*Coleoptera:* Scarabaeidae and Geotrupidae) of North Carolina cattle pastures and their implications for pasture improvement. Master's Thesis, North Carolina State University

Blaser P, Zimmermann S, Luster J, Shotyk W (2000) Critical examination of trace element enrichments and depletions in soils: As, Cr, Cu, Ni, Pb, and Zn in Swiss forest soils. Sci Total Environ 249:257–280

Cook DF, Dadour IR, Alis DN (1995) Effect of diet on the excretion profile of ivermectin in cattle faeces. Int J Parasit 26:291–295

Dadour IR, Cook DF, Hennessy D (2000) Reproduction and survival of the dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) exposed to abamectin and doramectin residues in cattle dung. Environ Entomol 29:1116–1122

Durhan EJ, Lambright CS, Makynen EA, Lazorchak J, Hartig PC, Wilson VS, Gray LE, Ankley GT (2006) Identification of metabolites of trenbolone acetate in androgenic runoff from a beef feedlot. Environ Health Perspect 114(S-1):65–68

Environment Canada (1993) Priority substances list report: arsenic and its compounds, EN40-215/14E. Environmental Canada, Ottawa

Floate KD, Wardhaugh KG, Boxall ABA, Sherratt TN (2005) Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. Annu Rev Entomol 50:153–179

Garbarino JR, Bednar AJ, Rutherford D, Beyer RS, Wershaw RL (2003) Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ Sci Technol 37:1509–1514

Grafe M, Eick MJ, Gross PR, Saunders AM (2002) Adsorption of arsenate and arsenite on ferrihydrite in the presence and absence of dissolved organic carbon. J Environ Qual 31:1115–1123

Gupta G, Charles S (1999) Trace elements in soils fertilized with poultry litter. Poultry Sci 78:695–698

Han FX, Kingery WL, Selim HM, Gerard PD (2000) Accumulation of heavy metals in a long-term poultry waste-amended soil. Soil Sci 165:260–268

Jones FT (2007) A broad view of arsenic. Poult Sci 86:2–14

Kennedy G, Cannavan A, McCracken RJ (2000) Regulatory problems caused by contamination, a frequently overlooked cause of veterinary drug residues. J Chromatogr A 882:37–52

Kolok AS, Snow DD, Kohno S, Sellin MK, Guillette LJ Jr (2007) Occurrence and biological effect of exogenous steroids in the Elkhorn River, Nebraska, USA. Sci Total Environ 388:104–115

Krishnamurti GSR, Naidu R (2002) Solid-solution speciation and phytoavailability of copper and zinc in soils. Environ Sci Technol 36:2645–2651

Madsen M, Overgaard Nielsen B, Holter P, Pedersen OC, Brøchner Jespersen J, Vagn Jensen K-M, Nansen P, Grïnvøld J (1990) Treating cattle with ivermectin: effects on the fauna and decomposition of dung pats. J Appl Ecol 27:1–15

Makris KC, Quazi S, Punamiya P, Sarkar D, Datta R (2008) Fate of arsenic in swine waste from concentrated animal feeding operations. J Environ Qual 37:1626–1633

Marin AR, Pezeshki SR, Masscheleyn PH, Choi HS (1993) Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. J Plant Nutr 16:865–880

Mortensen GK, Strobel BW, Hansen HCB (2006) Degradation of zearalenone and ochratoxin A in three Danish agricultural soils. Chemosphere 62:1673–1680

Nachman KE, Graham JP, Price LB, Silbergeld EK (2005) Arsenic: a roadblock to potential animal waste management solutions. Environ Health Perspect 113:1123–1124

Nielsen FH (1998) Ultratrace elements in nutrition: current knowledge and speculation. J Trace Elem Exp Med 11:251–274

Rabiet M, Togola A, Brissaud F, Seidel J-L, Budzinski H, Elbaz-Poulichet F (2006) Consequences of treated water recycling as regards pharmaceuticals and drugs in surface and ground waters of a medium-sized Mediterranean catchment. Environ Sci Technol 40:5282–5288

Russell R, Paterson M (2007) Zearalenone production and growth in drinking water inoculated with *Fusarium graminearum*. Mycol Progress 6:109–113

Rutherford D, Bednar AJ, Garbarino JR, Needham R, Staver KW, Wershaw RL (2003) Environmental fate of roxarsone in poultry litter. Part II. Mobility of arsenic in soils amended with poultry litter. Environ Sci Technol 37:1515–1520

Schiffer B, Daxenberger A, Meyer K, Meyer HHD (2001) The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environ Health Perspect 109:1145–1151

Smith DJ (2000) Total radioactive residues and clenbuterol residues in swine after dietary administration of [14C]clenbuterol for seven days and preslaughter withdrawal periods of zero, three, or seven days. J Anim Sci 78:2903–2912

Stark JD (2005) A review and update of the report "Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene" 1999 by Travis R. Glare and Maureen O'Callaghan. New Zealand Ministry of Health, Wellington. http://www.smsl.co.nz/biosecure/NZB/Literature/s-methoprene-update.pdf. Accessed 8 Aug 2008

Stolz JF, Perera E, Kilonzo B, Kail B, Crable B, Fisher E, Ranganathan M, Wormer L, Basu P (2007) Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by *Clostridium* species. Environ Sci Technol 41:818–823

Svendsen TS, Sommer C, Holter P, Grønvold J (2002) Survival and growth of *Lumbricus terrestris* (Lumbricidae) fed on dung from cattle given sustained-release boluses of ivermectin or fenbendazole. Eur J Soil Biol 38:319–322

Tyndale-Bisoce M (1996) Australia's introduced dung beetles: original releases and distributions. CSIRO entomol tech report no. 62

Wardhaugh KG (2005) Insecticidal activity of synthetic pyrethroids, organophosphates, Insect growth regulators, and other livestock parasiticides: an Australian perspective. Environ Toxicol Chem 24:789–796

Waterhouse DF, Sands DPA (2001) Classical biological control of arthropods in Australia. Monograph 77Australian Center for International Agricultural Research, Canberra, Australia

Wilken R-D, Ternes TA, Th H (2000) Pharmaceuticals in sewage, surface and drinking water in Germany. In: Deininger RA, Literathy P, Bartram J (eds) Security of public water supplies. Kluwer, Dordrecht, pp 227–240

Chapter 14
Environmental Impact and Risk of CAFOs

Amy Pruden and Laurence Shore

Abstract Although effects of compounds generated from CAFOs on fish and other aquatic organisms have been reported, the unanswered question is if the populations are affected. For the pharmaceuticals, the question is if non-target organism populations are seriously affected, in particular the dung beetle which has positive ecological effects over large areas. The antibiotics are of special concern for the emergence of antibiotic resistance strains. On the other hand, the animals used in domestic husbandry are genetically very similar. For example, all of the cows in Israel are sired from about ten bulls and they are raised in essentially the same 300 cow units. These animals breathe the same and eat many of the same foods and drink the same water as humans. They therefore can be our first line of defense in detecting environmental disruptions. The CAFOs can also be a valuable way of disposing of waste materials from many industries, especially the alcoholic beverages (molasses) and citrus industry (fruit grinds). Poultry manure can be silaged and is a good source of protein and bulk for cattle. Furthermore, the manure produced has become a valuable commodity as interest in organic farming grows and the price of inorganic fertilizers increases.

14.1 Introduction

It is apparent that risk assessment means different things to scientists and the general public. The general public cannot be expected to comprehend the incredible analytic power of the instruments available to the analytical chemist and the molecular biologist. Values such as ppm, ppb and ppt are virtually meaningless to a public that just hears that the scientist has found a substance in the food, water or soil that is carcinogenic or perilous to some species of wildlife. Regulatory authorities and

A. Pruden (✉) and L. Shore
Via Department of Civil & Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA, 24061, USA
e-mail: apruden@vt.edu

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_14, © Springer Science+Business Media, LLC 2009

legislatures in particular find it easiest to demand zero levels of putatively toxic compounds, a value that is unrealistic and meaningless at the molecular level. Often there is also a lack of appreciation even on the part of scientists as to the limitations of the scientific method. Applied to large systems such as ecosystems, the tools to accurately describe what is transpiring do not exist, e.g., predicting the weather. However, like most things in real life, decisions need to be made when only a small portion of the necessary data is available and extrapolation must be used. This leads to two approaches to risk assessment: cost/benefit ratio and the precautionary principle. Although the precautionary principle, i.e., any evidence that something may be toxic is sufficient to justify banning it (better safe than sorry) is generally associated with the EU's approach and cost/benefit (the cure may be worse that the bite) and a definitive data base (if it is not broken, do not fix it) with the American approach, regulatory agencies on both continents use a mix of the two approaches to determine risk assessment. The methodology of risk assessment of pharmaceuticals in the environment has been described in great detail in Kümmerer's *Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks*, particularly in the chapters by Huggert et al. (2004) and Webb (2004). Issues of risk assessment specifically focusing on antimicrobial use in agriculture have recently been reviewed by Vose (2006) in "Antimicrobial Resistance in Bacteria of Animal Origin." In an earlier chapter in the same book the need for more extensive monitoring and surveillance of antimicrobial resistance in animal agriculture is particularly lamented (McEwen et al. 2006).

Few studies have measured the impact of CAFOs on the aquatic environment in the absence of human sewage effluent contamination or runoff from agricultural croplands. Only two studies of cattle feedlot effluents have been reported – one on the impact on one species of fish and one on the effect on a turtle. There has been only one study on the effect of runoff from cattle grazing on 19 species of fish. There have been incidental studies on the impact of manure fertilization from CAFOs on soil ecology (8.3) and the effect of ivermectin on grassland ecology (13.3). In terms of antibiotic resistance, there are a handful of studies available that have linked antibiotic resistant infections in humans with antibiotic use in agriculture. Again, as is the case with hormones and other pharmaceuticals, it is difficult to separate effects of human use with agricultural use and it is particularly challenging to conclusively identify animal origins for antibiotic resistant diseases in humans.

14.1.1 Impact of Effluent from Cattle Feedlots on One Species of Fish

Samples in Nebraska, USA, were taken from three sites: intensive feedlot concentration, combined grazing and agricultural and a reference site. The parameters measured were the effects on fathead minnow (*Pimephales promelas*; morphological and gonadal steroidogenesis in vitro) and cells transfected with AR. It was found that (1) Male fish were demasculinized (having lower testicular testosterone synthesis, altered head morphometrics, and smaller testis size); (2) females were defeminized, as evidenced by a decreased estrogen:androgen ratio of in vitro steroid hormone synthesis; (3) there were potent androgenic responses from the feedlot effluent. There were no overt signs of feminization or masculization of the fish. The putative agents were trenbolone or fecal steroids but the results were not conclusive. In a parallel study, the androgenic and estrogenic activity in sensitive cell lines and the concentrations of E1, 17α-estradiol, E2, TbOHa and TbOHb were determined. Estrone was present 1-2 ng/l at 2 of the 6 sites tested, one in effluent and one in runoff from an agricultural area. Maximal estrogen concentrations were 8.5 ng/l E1, 3.8 ng/l 17α-estradiol and 3.2 ng/l E2. The levels of trenbolone and its metabolites were well below 1 ng/l. Therefore the androgenic and estrogenic responses observed in the cell lines were largely unexplained (Orlando et al. 2004; Soto et al. 2004).

14.1.2 Impact of Effluent from Cattle Feedlots on One Species of Turtle

The effects on *Chrysemys picta* of effluent from a feedlot containing 300 cattle (100 adults) on two sequentially placed ponds below the feedlot. The water contained 1–2 ng/l E2 which did not account for the majority of estrogen compounds present in the pond water. It was found that vitellogenin increased significantly in the female turtles (less than two fold) but male and juveniles were unaffected. The affected turtles were not examined for any morphological or histological parameters (Irwin et al. 2001).

14.1.3 Impact of Effluent from Rangeland on 19 Species of Fish

A survey of 19 species of fish present in a catchment area primarily draining a cattle pasture did not show any evidence of skewed sex ratio (Krotman 2004; Table 14.1). The maximum levels of hormones observed in the runoff streams were 6–8 ng/l of E or T and the maximum EE was 1.8 ng/l. The grazing intensity of the cattle was between 0.3 and 0.6 cows/ha. In the streams on the date of sampling, both T and E levels were below 3 ng/l and the EE was below level of detection (>0.5 ng/l).

However, in a nearby stream that had a rich variety of species (13 species), most species were identified as having a skewed sex ratio. Of particular concern was *Acanthobrama lissneri,* whose habitat is unique to the area. In this stream, measurable concentrations of ethinylestradiol (0.6–0.8 ng/l) were found. The source of the ethinylestradiol appeared to be from the large number of swimmers in the small stream rather than contamination with sewage effluent.

Table 14.1 Species of fish examined for sex ratio in cattle pasture drainage area with no observable affect. Fish observed at reference site with sex reversal are indicated with an *r*

Family	Species	
Cyprinidae	*Acanthobramalissneri*	*r*
	Barbus longiceps	*r*
	Barbus canis	*r*
	Garra rufa	*r*
	Cyprinus carpio	*r*
	Hemigrammocapoeta nana	*r*
	Capoeta damascina	
	Pseudophoxinus kervillei	
	Capoeta X Barbus longiceps	
Cichlidae	*Sarotherdon galilaeus*	*r*
	Oreochromis hybrid	*r*
	Tilapia zillii	*r*
	Astatotilapia flaviijosephi	*r*
Cyprinodontidae	*Aphanius mento*	*r*
Poeciliidae	*Gambusia affinis*	*r*
	Xiphophorus helleri	
	Molliensia velifera	*r*
Salmonidae	*Oncorhyncus mykiss*	
Balitoridae	*Nemacheilus spp*	

14.1.4 Links between VRE Infections in Humans and Avoparcin Use in the EU

Probably the most publicized and well-studied association between antibiotic use in animals and resistant infections in humans is the case of avoparcin use in broiler chickens in Europe and VRE infections in humans. In 1993, the first non-human reservoirs of vanA vancomycin-resistant *Enterococcus faecium* (VREF) were identified in the United Kingdom (Bates et al. 1993). Subsequent investigations in Germany and Denmark led to similar findings. Because of the importance of vancomycin as a last-resort drug, avoparcin, which is also a glycopeptide, was subject to an EU-wide ban in 1995. Following the ban, sharp declines in glycopeptide resistance among enterococci isolated from pigs and chickens has been observed, though resistance in pigs did not drop off until tylosin was also banned (Aarestrup et al. 2001). Though a reduction in carriage of VRE among healthy adults has been observed, there has not been a measurable decrease in actual VRE infection (Klare et al. 1999; van den Bogaard et al. 2000). Interestingly, avoparcin was never approved in the U.S., and VRE carriage among livestock has remained quite low (Simjee et al. 2006). However, some have paralleled the current situation in the U.S. with increases in streptogramin-resistant *E. faecium* (SREF) associated with virginamycin (Donabedian et al. 2003) to what has been experienced in the EU with respect to avoparcin.

14.1.5 Enrofloxacin Use in Poultry and Fluoriquinolone-Resistant *Campylobacter* in the USA

In 2005, a court ruling banned the use of enrofloxacin, a fluoroquinolone, in poultry. This was because of substantial evidence of a link between the drug use in chickens and antibiotic-resistant *Campylobacter* infections in humans. *Campylobacter* is a pathogen affecting both humans and animals, primarily causing gastrointestinal illness, and is the most commonly reported bacterial cause of foodborne infection in the US. The logic of the ruling was basically as follows: (1) *Campylobacter* negatively impacts the health of over 1 million people annually; (2) Poultry is a major source of *Campylobacter* infections; (3) The use of enrofloxacin (Endtz et al. 1991) results in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter* and therefore (4) fluoroquinolone-resistant *Campylobacter* in poultry can be transferred to humans and "can contribute to" fluoroquinolone-resistant *Campylobacter* infections in humans (USFDA 2006). This was a highly controversial ruling, requiring a 5 year court battle. However, a post-hoc risk assessment bolstered the case against enrofloxacin. A study conducted by the US-FDA Center for Veterinary Medicine (CVM 2001) determined that in 1998, 8,678 U.S. citizens acquired floroquinolone-resistant *Campylobacter* infections from chickens and received fluoroquinolone antibiotics as the first line of treatment.

14.1.6 *Salmonella enterica* serovar Typhimurium phage type DT104 (*Salmonella* DT104)

Multiple-antibiotic resistant *Salmonella enterica* serovar Typhimurium phage type DT104 (*S. enterica* DT104) has emerged as a global health threat. Human infections with multidrug-resistant DT104 isolates have been associated with the consumption of chicken, beef, pork, sausages and meat paste (Wall et al. 1995). A unique aspect of *S. enterica* DT104 is that it carries the multiple-antibiotic resistance genes on the chromosome, rather than on a plasmid. It is considered that this may allow the organism to retain its ability to resist antibiotics, even in the absence of antibiotic selection pressure. It is not clear from where exactly this pathogen originated, but it was first identified in the UK in seagulls and cattle in 1984 and has since been found worldwide. One of the leading theories is that the origin of this strain is from florfenicol use in aquaculture, which led to antibiotic resistance among at least two fish pathogens, *Photobacterium damselae* and *Vibrio anguillarum*, which subsequently passed the genes to *S. enterica* DT104 (Cloeckaert and Schwarz 2001; Angulo and Griffin 2000), although this is still subject to debate (Cloeckaert and Schwarz 2001). Because of their overall importance to human health and their documented passage to humans via the food chain, multi-drug resistant *Salmonella* play a central role in both the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and the U.S. National Antimicrobial Resistance Monitoring System (NARMS).

14.2 Risk Assessment: Water Sources

Although effects of hormones and pharmaceuticals have been well documented for various organisms, the effect on populations has not been done with the recent exception of Kidd et al. (2007) for EE for one of two species of minnows (Palace et al. 2006). This is because it is immensely difficult to measure population changes, especially in animals like fish which do not stay in any one area of their habitat for very long. In addition, the major demonstrable changes in fish populations are the results of habitat change (usually dams and canalization), over-fishing, alien fish species and non-native parasites. To demonstrate an additional change in population under these drastic circumstances is statistically extremely difficult. Furthermore, natural and synthetic hormones affect the endocrine system which is adaptable and can change the sensitivities and threshold levels to various hormones. Indeed, it is possible that rapid changes in the environment would be more disruptive than an established equilibrium. In any event due to the scant information available, there is insufficient evidence at present that fish or other aquatic organisms populations have been affected by hormones or pharmaceuticals released from CAFOs. Therefore the present environmental restrictions on the well-documented adverse effects of nutrients (nitrates, phosphates) should be of primary concern.

The pharmaceuticals and antibiotics used in domestic animal husbandry have not changed much in the last fifty years while the amount of food produced (exclusive of China) has just tripled (linear growth) which is about what would be expected from the increase in population (Speedy 2003). There of course has been a major shift to CAFOs as described in Chap. 1 but again this growth has been relatively gradual. The immediate negative environmental impacts of these CAFOs are fairly well defined and possible ecological disasters can be anticipated. Aquaculture also did not increase dramatically (again exclusive of China) in the last fifty years of the past century. However, as capture fishing can no longer meet the rising demand, there will be a major shift to aquaculture in the coming decades and this presents the potential for large scale ecological effects (Brugère and Ridler 2004). The assumption that lakes, rivers and bays dilute the compounds to the extent that wild aquatic species will not be affected are not substantiated (Chap. 12). Also, with respect to antibiotics, they are added directly to the water and therefore correct dosing is extremely challenging, if not impossible. Finally, the antibiotics added are in direct contact with the sediments and water column at the same concentration that the fish being treated are exposed to. Based on these observations, it may be logical to devote more resources to monitoring the effects of compounds used in aquaculture on the environment.

14.3 Risk Assessment: Soil

The micro-organisms in the soil exist in a constant state of chemical warfare (Harborne 1997). The soil organisms have therefore evolved a wide variety of defensive mechanisms and ability to digest thousands of secondary metabolites and naturally occurring compounds like the hormonal steroids. As discussed in Chaps. 8 and 11, the capacity of the soil biota to metabolize naturally occurring compounds is immense. Recent news was made of the capability of naturally-occurring soil bacteria to even "eat" antibiotics as a carbon and energy source (Dantas et al. 2008). Thus, even under worst case scenarios little estrogen or antibiotics reach the groundwater. The small amounts of testosterone and other steroid hormones reaching groundwater are well within the range of water organisms to metabolize or adapt to as these concentrations can be produced by the aquatic organisms themselves. The major and very significant exception to this is karst formations which do little to remove anthropogenic compounds like caffeine or steroid hormones produced by CAFOs and even allow the transmission of pathogens. It would therefore be a major concern to environmental compartments as well as human health to follow a policy of no release of excrement or effluents from CAFOs over karst formations or at least to have a rigorous manure management programs. However in general, manure management programs are more important to prevent nutrients from CAFOs from destroying streams and habitats in areas such as the Delmarva Peninsula than reduction of possible hormone or pharmaceutical effects, which would just be an added benefit.

While it may be expected that antibiotics from CAFOs could cause devastating shifts in the soil microbial ecology, generally this is not observed. One major reason for this is likely the fact that the concentrations of antibiotics in manure are well below therapeutic levels and that they typically degrade with time (De Liguoro et al. 2003). Also, it has been observed that the microbial community, though it may initially be disturbed, can quickly develop tolerance to antibiotics, especially in the presence of rich nutrients, such as is available in pig slurry (Schmitt et al. 2005). However, even though antibiotics present in manure are typically below the minimal inhibitory concentration (MIC) (Kemper et al. 2008), antibiotics have been observed to accumulate in soil above the MIC (Hamscher et al. 2002). In a study by Kotzerke et al. (2008) it was found that sulfadiazine had a negative and concentration-dependent impact on nitrogen cycling in soil.

There is a major problem due to our lack of knowledge of soil ecology and biodiversity. It can be seen by the case of the dung beetle in Australia (13.3), that even if one component of the dung ecology is missing, the effects can be seen over thousands of acres of land. However, since there is not enough information to predict where the ecological damage will occur due to pharmaceuticals and hormones generated from CAFOs, one cannot form a cost/benefit analysis on their effects. The agricultural community will likely just continue to develop ad hoc solutions as the problems becomes evident.

14.4 Potential Environmental Advantages of CAFOs

The negative aspects of CAFOs have been addressed in a recent report from The Pew Charitable Trusts and the Johns Hopkins Bloomberg School of Public Health (http://www.ncifap.org/reports/): (1) they may increase in the pool of antibiotic-resistant bacteria because of the overuse of antibiotics; (2) air quality problems; (3) the contamination of rivers, streams, and coastal waters with concentrated animal waste; (4) animal welfare problems, mainly as a result of the extremely close quarters in which the animals are housed; (5) and significant shifts in the social structure and economy of many farming regions throughout the country. These issues are currently the subject of numerous reports and commissions on how to deal with these problems especially in farm states ("The right to farm").

However, there are a number of environmental advantages of CAFOs which deserve consideration. (1) First line of defense. The animals used in domestic husbandry are genetically very similar. For example, all of the cows in Israel are sired from about ten bulls and they are raised in essentially the same 300 cow units. These animals breathe the same and eat many of the same foods and drink the same water as humans. They therefore can be our first line of defense in detecting environmental disruptions. (Of course, the same conditions of monoculture present the constant specter of rapid spread of emergent diseases.) (2) Disposal of waste materials. The CAFOs can be a valuable way of disposing of waste materials from many industries, especially the alcoholic beverages (molasses) and citrus industry (fruit grinds). Poultry manure can be silage and is a good source of protein and bulk for cattle (Fontenot et al. 1996). (3) Economy of scale. Large scale manure production allows the use of technologies which are not appropriate for small farms, e.g. production of biogas, on site composting (see next chapter). However, appropriate incentives are needed to encourage more rigorous centralized treatment of CAFO manure, oftentimes the capital costs are the burden of the farmer, who is already operating at a very slim profit margin.

In terms of antibiotic resistance, in general there has not been a major outcry to the extent that there has been in the human medical arena with respect to problems with treating animal diseases because of antibiotic resistance. However, some increases in resistance associated with antibiotic use among livestock pathogens have been observed. For example, penicillin was initially used to treat bovine mastitis caused by *S. aureus infection*, which subsequently led to the rise of penicillin-resistant *S. aureus* to the extent that the drug was no longer effective (Aarestrup and Jensen 1998). Similarly, resistance to macrolides among *S. hyicus* isolated from pigs has closely paralleled usage of macrolides in Denmark (Aarestrup and Jensen 2002). Pathogenic *E. coli* cause a wide range of infections, especially diarrhea, and are responsible for a high rate of mortality among chickens, pigs, goat, and sheep, and in particular juveniles of these species. Clear rises in *E. coli* resistance to several antimicrobials have been observed (White 2006). Despite documented increases in resistance among certain animal pathogens, in general most available antimicrobial agents can still be used to treat bovine mastitis (Aarestrup and Schwarz 2006) and other animal diseases. In Israel (where subtherapeutic use of antibiotics has been banned only in the last ten years), there have not been any changes in the dosage of antibiotics needed to treat mastitis or other cattle diseases for the last fifty years (Friedman S, personal communication). Thus, continued subtherapeutic antibiotic use in agriculture may not be as much of a concern to the animals themselves, especially considering a higher threshold for mortality that is considered acceptable for livestock as compared to humans.

Overall, elimination of any unnecessary antibiotic use, and in particular subtherapeutic use, is considered by many to be a prudent approach to extending the lifespan of existing antibiotics and protecting human and animal health. However, there may be a severe unintended environmental impact of this approach. Eliminating subtherapeutic use of antibiotics will result in slower weight gain and longer time to market, which likely translates into a higher waste production rate over a longer period of time. Since, as has been discussed above, nutrients and other general pollution issues with manure itself remain as major unsolved challenges, the potential for increased waste production should be considered with any proposed antibiotic ban. Aesthetic issues may also be a concern, whereas North Americans tend to prefer fatter meat products, a cultural adjustment to an antibiotic ban may be required. At present, Denmark provides the most extensive model of the consequences of eliminating subtherapeutic antibiotic use. A study of the impact on broiler chickens found that kilogram broilers produced per square meter and percent dead broilers in total were not affected by the discontinued use of antibiotic growth promoters. A fairly large-scale study of poultry in the US concluded that there is a slight production advantages to the use of growth promoting antibiotics, but that the benefits were economically off-set by the costs of the antibiotics (Graham et al. 2007). Both studies confirmed that the feed-conversion ratio did increase without antibiotic growth promoters, though marginally (up to 0.016 kg/kg in Denmark; Emborg et al. 2001). This supports the notion that there may be some environmental impact of ceasing subtherapeutic antibiotic use in terms of increased animal waste production.

Interestingly, a recent review and risk assessment suggested that the ban on subtherapeutic antibiotic use in Europe may have actually played a role in increasing antibiotic resistant infections in humans, in particular VRE (Phillips 2007). However, the conclusions of this study are currently the subject of heated debate (Hammerum et al. 2007; Phillips 2008).

References

Aarestrup FM, Jensen LB (1998) Development of penicillin resistance among *Staphylococcus aureus* isolated from bovine mastitis in Denmark and other countries. Microb Drug Resist 4:247–256

Aarestrup FM, Jensen LB (2002) Trends in antimicrobial susceptibility in relation to antimicrobial usage and presence of resistance genes in *Stapylococcus hyicus* isolated from exudative epidermitis in pigs. Vet Microbiol 89:83–94

Aarestrup FM, Schwarz S (2006) Antimicrobial resistance in *staphylococci* and *streptococci* of animal origin. In: Aarestrup FM (ed) Antimicrobial resistance in bacteria of animal origin, ASM Press, Washington, DC, pp 187–212

Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F (2001) Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal *enterococci* from food animals in Denmark. Animicrob Agent Chemother 45:2054–2059

Angulo FJ, Griffin PM (2000) Changes in antimicrobial resistance in *Salmonella enterica* serovar Typhimurium. Emerg Infect Dis 6:436–437

Bates J, Jordens Z, Selkon JB (1993) Evidence for an animal origin of vancomycin-resistant *enterococci*. Lancet 342:490–491

Brugère C, Ridler N (2004) Global aquaculture outlook in the next decades: an analysis of national aquaculture production forecasts to 2030. FAO Fisheries Circular, No. 1001, Rome, FAO

Cloeckaert A, Schwarz S (2001) Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* typhimurium DT104. Vet Res. 32:301–310

CVM (Center for Veterinary Medicine) (2001) U.S. FDA CVM: The human health impact of fluoroquinolone-resistant *Campylobacter* attributed to the consumption of chicken. Revised January 5, 2001

Dantas G, Sommer MOA, Oluwasegun RD, Church GM (2008) Bacteria subsisting on antibiotics. Science 320:100–103

De Liguoro M, Cibin V, Capolongo F, Halling-Sørensen B, Montesissa C (2003) Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere 52:203–212

Donabedian SM, Thal A, Hershberger E, Perri MB, Chow JW, Bartlett P, Jones R, Joyce K, Rossiter S, Gay K, Johnson J, Mackinson C, Debess E, Madden J, Angulo F, Zervos MJ (2003) Molecular characterization of gentamicin-resistant *enterococci* in the United States: evidence of spread from animals to humans through food. J Clin Microbiol 41:1109–1113

Emborg HD, Ersboll AK, Heuer OE, Wegener HC (2001) The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev Vet Med 50:53–70

Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP (1991) Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother 27:199–208

Fontenot JP, Ayangbile GA, Allen VG (1996) Potential for recycling animal wastes by feeding to reduce environmental contamination. In: Kornegay IT (ed) Nutrient management of food animals to enhance and protect the environment. CRC Press, Boca Raton FL pp 199–217

Food and Drug Administration Department of Health and Human Services (USFDA) (2006) Withdrawal of approval of the new animal drug application for enrofloxacin in poultry. Docket No 200N-1571

Graham JP, Boland JJ, Silbergeld E (2007) Growth promoting antibiotics in food animal production: an economic analysis. Pub Health Rep 122:79–87

Hammerum AM, Heuer OE, Lester CH, Agerso Y, Seyfarth AM, Emborg HD, Frimodt-Moller N, Monnet DL (2007) Comment on: withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. Intl J Antimicrob Agent 30:466–468

Hamscher G, Sczesny S, Höper H, Nau H (2002) Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal Chem 74:1509–1518

Harborne JB (1997) Recent advances in chemical ecology. Nat Prod Rep 14:83–98

Huggert DB, Ericson JF, Cook JC, Williams RT (2004) Plasma concentrations of human pharmaceuticals as predictors of pharmacological responses in fish. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn. Springer-Verlag, Heidelberg, pp 373–386

Irwin LK, Gray SL, Oberdorster E (2001) Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol. Aquat Toxicol 55:49–60

Kemper N, Färber H, Sklutlarek D, Krieter J (2008) Analysis of antibiotic residues in liquid manure and leachate of dairy farms in Northern Germany. Agric Water Manage 95: 1288–1292

Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW (2007) Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci USA 104:8897–8901

Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W (1999) Decreased incidence of van A-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist 5:45–52

Kotzerke A, Sharma S, Schauss K, Heuer H, Thiele-Bruhn S, Smalla K, Wilke BM, Schloter M (2008) Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. Environ Pollut 153:315–322

Krotman Y (2004) Biodiversity and community structure of Jordan system fishes and their relationship to the habitat structure and anthropogenic impact. M.Sc. Thesis, Department of Zoology, Tel Aviv University

McEwen SA, Aarestrup FM, Jordan D (2006) Monitoring of antimicrobial resistance in animals: principles and practices. In: Aarestrup FM (ed) Antimicrobial resistance in bacteria of animal origin, ASM Press, Washington DC, pp 397–413

Orlando EF, Kolok AS, Binzcik GA, Gates JL, Horton MK, Lambright CS, Gray Jr LE, Soto AM, Guilette LJ Jr (2004) Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environ Health Perspect 12:1–9

Palace VP, Wautier KG, Evans RE, Blanchfield PJ, Mills KH, Chalanchuk SM, Godard D, McMaster ME, Tetreault GR, Peters LE, Vandenbyllaardt L, Kidd KA (2006) Biochemical and histopathological effects in pearl dace (*Margariscus margarita*) chronically exposed to a synthetic estrogen in a whole lake experiment. Environ Toxicol Chem 25:1114–1125

Phillips I (2007) Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. Intl J Antimicrob Agent 30:101–107

Phillips I (2008) Withdrawal of growth-promoting antibiotics: comments on commentary. Intl J Antimicrob Agent 31:82–83

Schmitt H, Haapakangas H, van Beelen P (2005) Effects of antibiotics on soil microorganisms: time and nutrients influence pollution-induced community tolerance. Soil Biol Biochem 37:1882–1892

Simjee S, Jensen LB, Donabedian SM, Zervos MJ (2006) *Enterococcus*. In: Aarestrup FM (ed) Antimicrobial resistance in bacteria of animal origin. ASM Press, Washington DC, 397–413

Soto AM, Calabro JM, Prechtl NV, Yau AY, Orlando EF, Daxenberger A, Kolok AS, Guillette Jr LJ, le Bizec B, Lange IG, Sonnenschein C (2004) Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in eastern Nebraska, USA. Environ Health Perspect 112:346–352

Speedy AW (2003) Global production and consumption of animal source foods. J Nutr 133: 4048 S–4053S

Van den Bogaard AE, Bruinsma N, Stobberingh EE (2000) The effect of banning avoparcin on VRE carriage in The Netherlands. J Antimicrol Chemother 46:146–148

Vose D (2006) Risk assessment and its use in approval, licencing, and prudent use guidelines. In: Aarestrup MF (ed) Antimicrobial Resistance in Bacteria of Animal Origin. ASM Press, Washington, DC 415–424

Wall PG, Morgan D, Lamden K, Griffen M, Threlfall EJ, Rowe B (1995) A case control study of infection with an epidemic strain of multiresistant *Salmonella typhimurium* DT104 in England and Wales. Communicable Disease Report CDR Review 4 (1994): R130–R135

Webb SF (2004) A data based perspective on the environmental risk assessment of human pharmaceuticals III indirect human exposure. In: Kümmerer K (ed) Pharmaceuticals in the environment. Sources, fate, effects and risks, 2nd edn. Springer-Verlag, Heidelberg 373–386

White DG (2006) Antimicrobial Resistance in pathogenic *Escherichia coli* from Animals. In: Aarestrup FM (ed) Antimicrobial resistance in bacteria of animal origin. ASM Press, Washington DC 145–166

Chapter 15
Management Approaches to Dealing with Pharmaceuticals and Hormones from CAFOs

Laurence Shore and Amy Pruden

Abstract Hormones and most pharmaceuticals can be prevented from entering the environment by simple technologies. Composting is extremely effective in destroying all compounds tested to date. Similarly, silaging is effective in removing most compounds. Closing off the CAFO from the environment leaves the question of what to do with the huge amount of manure accumulated. Fortunately, the demand for organic fertilizers has risen in the last decade as the result of increased interest in organic farming and the rise in the cost of inorganic fertilizers. In addition, to composting, the material can be converted to biogas. Controlling run-off and other common sense best management practices are also likely to help prevent the spread of pharmaceuticals in the environment.

15.1 Introduction

The major problem of CAFOs is the release of nutrients into the environment, particularly nitrates. The excess nitrate and phosphorus damage water supplies making them hostile to aquatic species requiring oxygen and contaminating water supplies above the levels permitted by regulatory authorities. Pharmaceuticals and hormones contained in the effluents from CAFOs are not currently regulated; however, treatment of the effluent to acceptable levels of nitrate and other nutrients will likely also have beneficial impact on the treatment of most hormones and drugs.

L. Shore (✉) and A. Pruden
Departments of Endocrinology and Toxicology, Kimron Veterinary Institute,
POB 12, Bet Dagan, 50250, Israel
e-mail: shorel@int.gov.il

L.S. Shore and A. Pruden (eds.), *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations*, Emerging Topics in Ecotoxicology 1,
DOI 10.1007/978-0-387-92834-0_15, © Springer Science+Business Media, LLC 2009

15.2 Elimination at Source

The most direct management engineering approach would be to eliminate the problem of the pharmaceuticals before they reach the effluent. This is done by using environmentally degradable compounds such as is done in most modern CAFOs, e.g., elimination of the chlorinated organic insecticides which are persistent. Antibiotics represent a special case as the problem is not the residues of antibiotics but the resistant bacteria presumably produced. There is a consensus that discontinuing "unnecessary" use of antibiotics important for human medicine for growth promotion should be discontinued. For example, the fluoroquinolone vancomycin was widely used in broiler chickens in Europe and its elimination in the mid-1990s has led to a dramatic decrease in the carriage of vancomycin resistant enterococci (VRE) in animals and humans (van den Bogaard et al. 2000; Hammerum et al. 2007a), though actual decrease in human VRE infection is debatable (Phillips 2007; Cox and Ricci 2008; Hammerum et al. 2007b). Organic arsenics discussed in the previous chapter are also slated for elimination because, being an element, the proposed solutions of biogas or composting cannot reduce the concentration of total Arsenic.

One major problem with large scale banning of certain compounds is compliance. If a substantial loss in income results from not using a compound then the illegal use of the compounds will continue without any regulation. Such unregulated use can have disastrous consequences such as seen with human fatalities as the result of illegal clenbuterol residues in meat (Smith 2000). For an elimination program to be effective the user has to be convinced that no significant profit is lost and that there is a real demonstrable danger to human health or his immediate environment. For this reason, studies on the economic impact of eliminating sub-therapeutic antibiotic use in Europe have been closely watched. A study conducted in Sweden indicated that because of the elimination of sub-therapeutic antibiotics, animals became ill more frequently and thus therapeutic antibiotic use increased (Casewell et al. 2003). Increased diarrhea, weight loss and mortality due to *Escherichia coli* and *Lawsonia intracellularis* in early post-weaning pigs and clostridial necrotic enteritis in broiler chickens were the chief complaints. However, total antibiotic use still remained below previous levels, which could have overall economic advantages. In a recent study in the US, it was found that eliminating antibiotics in adult swine had no impact on health and that the overall effect was a cost savings, though antibiotics were still recommended for nursery pigs (McBride et al. 2008).

15.3 Reducing the Use by Good Management Practice

The use for pesticides and parasiticides can be reduced by good management practice (management practices for aquaculture are discussed in Sects. 12.3.7 and 12.4.5).

1. If the manure is removed weekly, the manure dries out before fly larvae can mature and dung beetles and earthworms do their tasks much better in moist rather than wet manure. If the manure is removed daily (e.g. while the cows are being milked) it can be composted in nearby compost sheds, which will destroy the drugs while making excellent compost and maintaining good bedding for the cows. However, the composting can be done over long time periods if moisture and aeration are maintained (Kahn et al. 2007). Composting completely destroys most organic compounds (Barker and Bryson 2002) and hormones (Personal observation).
2. Many farms overdose with parasiticides. This is in line with the tendency of producers in general to believe that more treatment is better. Good management uses agents when necessary by biological monitoring. Since every animal operation is different, this means educational programs to provide the necessary information to the farmers and ranchers.
3. Timing of application. Avoidance of treatment at the times most likely to damage non-target organisms, e.g. avoid treatment when dung beetle larva emerge in the Spring (Dadour et al. 2000).
4. Change the treatment in alternate years or seasons, e.g. for fly control alternate between organic phosphates and IGFs or for worm control rotate the type of avermectin used (Dadour et al. 2000).

15.4 Preventing the Effluents from Reaching the Environment

The second stage in management would be prevention of the materials reaching the environment. This is done by preventing any effluent from leaving the CAFOs by the use of concrete or plastic lined basins. For intensive grazing and feedlots, barrier strips can also be effective. Many jurisdictions forbid cattle from directly accessing streams or other water sources.

Based on the antibiotic studies of Davis et al. (2006) and Dolliver and Gupta (2008) we have learned that antibiotics will be present in runoff, both in the aqueous phase and adhered to sediments. Therefore, general best management practices for controlling runoff and sediment transport are likely to play a major role in keeping antibiotics onsite. This will also likely help control the transport of antibiotic resistant bacteria as well as fecal bacteria.

15.5 Destruction of Potential Environmentally Active Compounds

The third management stage would be to foster actual destruction of the compounds. Composting, silaging and biogas production would fall under this category. The assumption is that CAFOs generate more volume of effluent than can be used for fertilization since they are concentrated in small areas and the cost of transporting the material to other areas is prohibitive or restricted by geopolitical considerations. Also, composting provides a high quality soil amendment that enhances moisture and nutrient levels.

Composting has been demonstrated to be highly beneficial for the destruction of antibiotic compounds (Storteboom et al. 2007; Dolliver and Gupta 2008). According to Dolliver and Gupta, the half-lives for chlortetracycline, monensin, and tylosin in composted turkey litter were found to be 1, 17, and 19 days, respectively. However, no difference in degradation rate was found between stockpiling and composting. Storteboom and colleagues did note a slight advantage of composting over stockpiling in beef manure and estimated half-lives of tetracycline, oxytetracycline, and chlortetracycline ranging between 4–15 days, compared to 8–30 days for stockpiling. Mechanisms of antibiotic destruction during composting likely include microbial biodegradation and superficial exposure to UV light from the sun.

The effect of composting on antibiotic resistance is not entirely clear. In the Storteboom study above (2007) it was found that beef manure, which is regularly exposed to sub-therapeutic antibiotics, did have higher initial concentrations of tetracycline resistance genes than dairy manure, in which sub-therapeutic antibiotic use is prohibited. Thus approximately 4 months were required to attenuate tetracycline resistance in dairy manure and 6 months in beef manure, but ultimately the levels of resistance achieved were similar between the two. Interestingly, when antibiotics were spiked into horse manure and then composted, concentrations of tetracycline resistance genes actually increased before finally decreasing to near-initial levels. Compost is a dynamic system, in which heat is initially generated from aerobic degradation, followed by colonization of thermophiles, before finally stabilizing to ambient temperatures. Thus, the microbial ecology is likely to play a significant role in determining at which stage bacteria carrying resistance genes are eliminated.

Pei et al. (2007) also observed that antibiotic resistance genes could increase in concentration in response to certain conditions in dairy lagoon water. In this laboratory-scale study, antibiotics were spiked into dairy lagoon water and subjected to different treatments: aerated, anaerobic, high temperature (20°C), and low temperature (4°C). In particular, sulfonamide resistance genes increased in concentration and did not return to initial levels in most treatments, while tetracycline resistance genes did not increase as dramatically. In general, aerated high temperature treatment was found to be the most effective for treating both antibiotics and antibiotic resistance. Peak et al. (2007) found a similar result in on-farm lagoons and suggested that land-application of lagoon material should thus be avoided at all cost during the winter season. Again, it is likely that the same strategies that enhance the effectiveness of lagoons for the treatment of organics and nutrients are also likely to help in managing antibiotics and resistance.

There has been some concern that the presence of antibiotics in animal waste may hamper beneficial biogas production. Although initial attempts in biogas production were not successful, today well designed and maintained facilities are being promoted as a solution for CAFOs. At present, these facilities have been demonstrated to be viable for large CAFOs of dairy cows (at least 500 head) and swine (at least 2000 sow or feeder pigs). Large amounts of antibiotics in the effluent are not compatible with biogas production and cold temperatures reduce its effectiveness. The effectiveness of anaerobic digestion of these facilities in reducing hormones and pharmaceuticals has not been evaluated but would be in the same order as achieved by anaerobic digestion in standard sewage treatment plants. Therefore the benefit would be primarily of preventing the resultant solid from reaching water sources and their subsequent use as biofertilizers.

15.6 Utilization of the Manure with Concern for the Environment

The fourth management technique would be recycling or utilization. For centuries, manures have been used for fertilization and as a valuable biological resource providing necessary nutrients. For this purpose, manure management programs based on the nitrogen and phosphorus content of the manure have been developed by the EU and many US states like Maryland and Ohio. The manure can also be silaged with wheat or corn waste to provide nutrient in the form of feed. However, the recent experience with BSE in which waste from animals of the same species, has darkened the prospects of the potential the use of waste products of even heterologous species. (The homogenous species feeding of waste products was an extremely poor idea and contrary to good veterinary practice, if not just plain common sense.) This utilization is driven by economic considerations. With the increased demand for organic farming and the increase in cost of inorganic fertilizers, at some point the demand will exceed the supply. At this point, the manure and effluents stop being an economic burden requiring the farmer to pay for its removal to a commodity from which to make a profit.

Proactively implementing best management practices to control pharmaceuticals and hormones by composting or other means is likely to help the profitability of manure products, considering today’s market being driven largely by consumer perceptions. However, as duly noted by the recent Pew report, too much of the cost of manure treatment is born by the producer and too little by the sponsoring agribusiness that is making the lion-share of the profit. Clearly, policy is likely to play a key role not only in regulating use and treatment of hormones and antibiotics, but also ensuring that the burden is shared fairly, including potential increases in cost if the consumer is willing.

References

Barker AV, Bryson GM (2002) Bioremediation of heavy metals and organic toxicants by composting. ScientificWorldJournal 2:407–420

Casewell M, Friis C, Marco E, Mcmullin P, Phillips I (2003) The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52:159–161

Cox LA, Ricci PF (2008) Causal regulations vs. political will: Why human zoonotic infections increase despite precautionary bans on animal antibiotics. Environ Int 34:459–475

Dadour IR, Cook DF, Hennessy D (2000) Reproduction and survival of the dung beetle *Onthophagus binodis* (Coleoptera: Scarabaeidae) exposed to abamectin and doramectin residues in cattle dung. Environ Entomol 29:1116–1122

Davis JG, Truman CC, Kim SC, Ascough JC, Carlson K (2006) Antibiotic transport via runoff and soil loss. J Environ Qual 35:2250–2260

Dolliver H, Gupta S (2008) Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J Environ Qual 37:1227–1237

Hammerum AM, Heuer OE, Emborg HD, Bagger-Skjot L, Jensen VF, Rogues AM, Skov RL, Agerso Y, Brandt CT, Seyfarth AM, Muller A, Hovgaard K, Ajufo J, Bager F, Aarestrup FM, Frimodt-Moller N, Wegener HC, Monnet DL (2007a) Danish integrated antimicrobial in resistance monitoring and research program. Emerg Infect Dis 13:1632–1639

Hammerum AM, Heuer OE, Lester CH, Agersø Y, Seyfarth AM, Emborg HD, Frimodt-Moller N, Monnet DL (2007b) Comment on: withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. Int J Antimicrob Agents 30:466–468

McBride W, Key N, Mathews KH Jr (2008) Subtherapeutic antibiotics and productivity in U.S. hog production. Rev Agr Econ 30:270–288

Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW (2007) Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. Environ Microbiol 9:143–151

Pei R, Cha J-M, Carlson KH, Pruden A (2007) Biological treatment of antibiotic resistance genes (ARG) in dairy lagoon water. Environ Sci Technol 41:5108–5113

Phillips I (2007) Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. Int J Antimicrob Agents 30:101–107

Smith DJ (2000) Total radioactive residues and clenbuterol residues in swine after dietary administration of [14C]clenbuterol for seven days and preslaughter withdrawal periods of zero, three, or seven days. J Anim Sci 78:2903–2912

Storteboom HN, Kim SC, Doesken K, Davis J, Carlson KH, Pruden A (2007) Response of Antibiotics and Resistance Genes to High-Intensity and Low-Intensity Manure Management. J Environ Qual 36:1695–1703

van den Bogaard AE, Bruinsma N, Stobberingh EE. (2000) The effect of banning avoparcin on VRE carriage in The Netherlands. Journal of Antimicrobial Chemotherapy. 46:146–148

Index