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Analysis of hormonal veterinary medicines and steroid oestrogens in agricultural run-off

Science Report: SC040072/SR

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This report is the result of research commissioned and funded by the Environment Agency's Science Programme.

Published by:
Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD
Tel: 01454 624400 Fax: 01454 624409
www.environment-agency.gov.uk

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Dissemination Status:
Publicly available

Keywords:
Hormones, veterinary medicines, agriculture, endocrine disruption

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Science Project Number:
SC040072

Product Code:
SCHO0706BKZB-E-P

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Steve Killeen

Head of Science

Executive summary

Veterinary medicines and natural steroids are released into the environment from agricultural sources, primarily through excretion from livestock. However, it is not known whether these compounds reach surface waters via run-off from agriculture and whether they subsequently contribute to endocrine disruption in the aquatic environment.

For this reason, the Department for Environment, Food and Rural Affairs (Defra) funded a project to establish whether intensive livestock rearing in the UK may be contributing to oestrogenic and/or androgenic activity in headwater streams. Sampling was carried out at eleven 'worst-case' agricultural sites where run-off from either cattle or pig farming was expected. Oestrogenic and androgenic activity was determined using *in vitro* yeast oestrogen and androgen screens and reported in Matthiessen *et al*. (2005).

Duplicate samplers were used at ten of these sites and passed on to the Environment Agency for chemical analysis, to determine if any steroid oestrogens were present along with selected veterinary medicines. None of the veterinary medicines monitored for were detected at concentrations above the limits of detection. However, the natural steroid oestrogens, oestrone and 17 beta oestradiol, were found to be present.

Greater concentrations of natural steroid oestrogens were measured at sites downstream of the farms compared to upstream sites at several of the locations studied. This suggests that intensive livestock rearing can and does release oestrogens into surface waters. However, some study sites had greater concentrations of steroid oestrogens at upstream sites, although the reason for this was unclear.

Finally, concentrations of oestrone and the total oestrogenic toxic equivalents were detected above levels of concern, suggesting that these compounds could potentially be exerting adverse effects on aquatic communities.

Contents

1 Introduction

Veterinary medicines and natural steroids are released into the environment from agricultural sources, primarily through excretion from livestock. It is not known whether these compounds reach surface waters via run-off from agriculture and whether they subsequently contribute to endocrine disruption in the aquatic environment (Boxall *et al*., 2002). Studies in the USA indicate that some steroids from agricultural sources may be present in headwater streams at concentrations of concern (Kolodziej *et al*., 2004), but the situation in the UK has not yet been established.

For this reason, the Department for Environment, Food and Rural Affairs (Defra) funded a project to establish whether intensive livestock rearing in the UK may be contributing to oestrogenic and/or androgenic activity in headwater streams. Passive sampling devices known as Polar Organic Chemical Integrative Samplers (POCIS) were deployed at eleven 'worst-case' agricultural sites where run-off from either cattle or pig farming was expected. Oestrogenic and androgenic activity was determined using *in vitro* yeast oestrogen and androgen screens and reported in Matthiessen *et al*. (2005).

Duplicate POCIS samplers were deployed at ten of these sites and supplied to the Environment Agency for chemical analysis, to determine if any steroid oestrogens were present in the sample. Results of these analyses were passed on to the Defra project consortium and reported with the results of the bioassays (Matthiessen *et al*., 2005).

In addition to steroid oestrogens, selected veterinary medicines were also analysed for. This information was used to compare the relative importance of any inputs from natural or medicinal hormone sources. Information on the number of livestock, hormonal veterinary medicine products used, proximity to watercourses, weather conditions and slurry spreading on each farm was also collated to aid interpretation.

This report presents the results of the steroid oestrogen and veterinary medicine analyses. Where possible, the results are converted into environmental concentrations and the possible impacts on aquatic ecosystems discussed. Finally, recommendations are made for future work in this area.

2 Methods

Eleven farms were selected to investigate the risk to the aquatic environment of endocrine disrupters from agriculture (Matthiessen *et al*., 2005). The farms were chosen to represent worst-case situations where small streams, uncontaminated with non-farm wastes, flowed through intensive livestock farms. Although one pig unit was included, the study mainly focused on cattle farms, given that pregnant cattle have been shown to excrete far more oestrogen than other types of livestock (Blok and Wösten, 2000; Johnson, Williams and Matthiessen, 2005). A full description of the method of farm selection is given in the Defra report.

Sampling took place between November 2004 and January 2005. Where possible, upstream and downstream sampling points were identified. In order to maximise the chances of detecting oestrogenic activity, monitoring was undertaken using a passive sampling technique. Passive samplers offer several advantages over conventional spot samples (Huckins *et al*., 1993), including:

- they only sample the dissolved load, which can be taken to be the bioavailable fraction;
- they accumulate contaminants over time, so that peak events are not missed;
- because they accumulate contaminants over time, the analytical limit of detection is more likely to be reached.

The type of passive sampler deployed was a Polar Organic Contaminant Integrative Sampler or POCIS (Alverez *et al*., 2004), which is designed to accumulate polar compounds such as steroid oestrogens. Duplicate POCIS deployed at ten of the sites were supplied to the Environment Agency for chemical analysis. The deployment dates and durations at these ten sites are given in Table 2.1.

Table 2.1 Timing of POCIS deployment and location of sampling points

Farm	Date of sample collection	Deployment duration (days)	Position	Approx. distance between points (m)
1	24/12/04	33	Upstream	
	24/12/04	33	Downstream	1000
2a[§]	11/12/04	31	Upstream	
	11/12/04	31	Downstream	900
2b	25/1/05	45	Upstream	
	25/1/05	45	Downstream	900
3a[§]	11/12/04	31	Upstream	
	11/12/04	31	Downstream	700
3b	25/1/05	45	Upstream	
	25/1/05	45	Downstream	700
4	17/12/04	32	Downstream	
6	21/12/04	39	Downstream	
	21/12/04	39	River control	
8	21/12/04	42	Upstream	
	21/12/04	42	Downstream	500
9	22/12/04	43	Upstream	
	22/12/04	43	Downstream	700
11	22/12/04	43	Upstream	
	22/12/04	43	Downstream	1300
13	25/1/05	73	Upstream	
	22/12/04	40	Downstream	600
14a[§]	6/1/05	42	Upstream	
	6/1/05	42	Downstream	100
14b	24/1/05	18	Downstream	

[§]Site was sampled twice. The earlier sample is referred to as a and the later sample as b throughout the text.

2.1 POCIS analysis

POCIS discs with an Oasis HLB sorbant were used, as this sorbant accumulates very polar compounds. After retrieval, the contents of the POCIS were transferred with methanol into individual glass chromatography columns. Sorbant analytes were solvent extracted using 40 ml of methanol and the collected eluate evaporated by rotary evaporation in a TurboVap system to one millilitre. The eluate was then made up to two millilitres using methanol and split into two equal aliquots. As the POCIS contained 200 mg sorbant, each aliquot represented 100 mg sorbant. One aliquot was analysed for steroid oestrogens, and the second for hormonal veterinary medicines.

2.1.1 POCIS steroid oestrogen analysis

Following the addition of internal standards, the extracts were concentrated under a nitrogen stream to facilitate a solvent exchange prior to being fractioned using size exclusion chromatography (gel permeation). The fraction containing the oestrogens

was collected and then concentrated prior to a further solvent exchange to facilitate an aminopropyl cartridge clean-up step.

The resultant extract was then taken to dryness and immediately mixed with a buffer solution, followed by a dansyl chloride solution. This mixture was then heated for a short period to aid the reaction, and cooled and transferred to a vial for analysis. Analysis was carried out using LC-MS/MS with photoionisation interface. Oestrone (E1), 17 beta oestradiol (E2) and 17 alpha ethinyl oestradiol (EE2) were quantified using an internal standard method with calibration against absolute standard solutions. Reporting limits of 1.0 ng/g for EE2 and 1.5 ng/g for E1 and E2 were set.

2.1.2 POCIS hormonal veterinary medicine analysis

Although no longer used as growth promoters in the UK, hormones are still used to induce or suppress oestrus, for example. Information on the active ingredients approved to treat cattle and pigs was obtained from the Veterinary Medicines Directorate (VMD, Table 2.2). Boxall *et al*. (2002) reported that the most used active substances across all livestock were altrenogest and progesterone in 2000.

Table 2.2 Active ingredients of hormonal veterinary medicines used to treat cattle and pigs

Active ingredient	Hormone type	Used for cattle	Used for pigs
Alfaprostol	Prostaglandin	Yes	Yes
Altrenogest	Progestagen		Yes
Buserelin	Gonadotrophin releasing hormone	Yes	
Carbetocin	Anterior pituitary	Yes	Yes
Cloprostenol	Prostaglandin	Yes	
Dinoprost	Prostaglandin	Yes	Yes
Etiproston	Prostaglandin	Yes	
Follicle stimulating hormone (FSH)	Gonadotrophin	Yes	Yes
Gonadorelin	Gonadotrophin releasing hormone	Yes	
Gonadotrophin	Gonadotrophin	Yes	Yes
Human chorionic gonadotrophin	Gonadotrophin	Yes	
Lecirelin	Gonadotrophin releasing hormone	Yes	
Luteinising hormone (LH)	Gonadotrophin	Yes	
Luprostiol	Prostaglandin	Yes	Yes
Oestradiol benzoate	Steroid	Yes	
Oestradiol valerate	Steroid	Yes	
Oxytocin	Anterior pituitary	Yes	Yes
Pregnant mare serum gonadotrophin (PMSG)	Gonadotrophin	Yes	Yes
Progesterone	Steroid	Yes	

The Environment Agency's National Laboratory Service (NLS) did not have analytical methods for any of these compounds. Within the timescales of the project, it was estimated that methods could be developed for six of these compounds; carbetocin,

cloprostenol, dinoprost, oestradiol valerate, oxytocin and progesterone. Selection of these compounds was based on the estimated ease of method development and sourcing of standards. Although a reference material was sourced for oxytocin, a method that gave an adequate response could not be developed and so no analysis was made for this compound.

Extracts were analysed on an Agilant 1100 HPLC system fitted with a Phenomenex 5.0 µm phase Phenyl-Hexyl column 150 mm * 4.6 mm i.d., interfaced to a mass spectrometer set in positive/negative electrospray mode. Quantification was achieved using calibration against standard solutions. Reporting limits were set at 5.0 µg/g for dinoprost, cloprostenol and oestradiol valerate, 20 µg/g for progesterone and 100 µg/g for carbetocin.

2.2 Farm information

Anonymised site data, collected as part of the Defra project, was supplied to the Environment Agency in order to aid interpretation of the chemical analysis. Supplementary information was also obtained on the hormonal veterinary medicines used on each farm during the study period and for the preceding 12 months. As all veterinary hormones are prescription-only treatments, a definitive list of the hormones used at each farm should be available from the vet. Information was gathered by the Centre for Ecology and Hydrology and Cambridge Environmental Assessments during face-to-face interviews with the farmers.

3 Results and observations

3.1 POCIS results

The results of the steroid oestrogen analyses are shown in Table 3.1. EE2 (17 alpha ethinyl oestradiol) was not detected in any sample. Greater concentrations of E1 (oestrone) were measured on the POCIS than E2 (17 beta oestradiol).

Table 3.1 Steroid oestrogen analysis results in mass per gram sorbant (ng/g)

Farm	Position	E1	E2	EE2
1	Upstream	1.94	<1.5	<1
	Downstream	44.8	4.99	<1
2a	Upstream	3.24	<1.5	<1
	Downstream	36.5	4.72	<1
2b	Upstream	2.97	<1.5	<1
	Downstream	29.6	4.14	<1
3a	Upstream	2.88	<1.5	<1
	Downstream	65.2	7.4	<1
3b	Upstream	2.17	<1.5	<1
	Downstream	97.9	11.4	<1
4	Downstream	134	7.44	<1
6	Downstream	1.8	<1.5	<1
	River control	5.68	<1.5	<1
8	Upstream	11.6	<1.5	<1
	Downstream	3.54	<1.5	<1
9	Upstream	24.5	2.06	<1
	Downstream	17.1	<1.5	<1
11	Upstream	79.5	17.3	<1
	Downstream	50.2	4.5	<1
13	Upstream	19.3	2.72	<1
	Downstream	7.28	1.62	<1
14a	Upstream	5.31	<1.5	<1
	Downstream	8.49	<1.5	<1
14b	Downstream	2.5	<1.5	<1

Passive sampling results in mass per device can be converted into a time weighted average (TWA) concentration. This is the average concentration to which the passive sampler was exposed over the sampling period. The TWA concentration can be calculated as C_S/R_St where C_S is the mass on the passive sampler, R_S is the uptake rate and t is the deployment time in days (Alverez *et al*., 2004). In order to calculate the TWA, calibration data is needed to determine the rate of uptake of each compound of interest. Matthiessen *et al*. (2005) did some preliminary calibration work to determine the uptake rate of E2 on POCIS, which was found to be 0.09 litres per day at 10°C. This uptake rate has been used for both E1 and E2 (Table 3.2).

Concentrations of E1 were also converted to E2 equivalents on the basis that E1 * 0.333 = E2 (Thorpe *et al*., 2003) and the total E2 equivalents for each site summed.

Table 3.2 Steroid oestrogen analysis results converted to estimated TWA concentrations and to E2 equivalents (ng/l)

Farm	Position	E1	E2	E2 equivalents
1	Upstream	0.13	0	0.04
	Downstream	3.02	0.34	1.35
2a	Upstream	0.23	0	0.08
	Downstream	2.62	0.34	1.21
2b	Upstream	0.15	0	0.05
	Downstream	1.46	0.20	0.69
3a	Upstream	0.21	0	0.07
	Downstream	4.67	0.53	2.09
3b	Upstream	0.11	0	0.04
	Downstream	4.83	0.56	2.17
4	Downstream	9.31	0.52	3.62
6	Downstream	0.1	0	0.03
	River control	0.32	0	0.11
8	Upstream	0.61	0	0.20
	Downstream	0.19	0	0.06
9	Upstream	1.27	0.11	0.53
	Downstream	0.88	0	0.29
11	Upstream	4.11	0.89	2.26
	Downstream	2.59	0.23	1.09
13	Upstream	0.59	0.08	0.28
	Downstream	0.4	0.09	0.22
14a	Upstream	0.28	0	0.09
	Downstream	0.45	0	0.15
14b	Downstream	0.31	0	0.10

Five veterinary medicines were measured for in each of the samples; carbetocin, cloprostenol, dinoprost, oestradiol valerate and progesterone. None of these compounds were present at concentrations above the limits of detection in any of the samples.

3.2 Farm information

Background information on each farm is shown below in Tables 3.3 and 3.4. Table 3.3 shows the basic information from each farm, whilst Table 3.4 shows the potential for excreta to reach the adjacent watercourses.

Table 3.3 Basic information on each farm

Farm	Environment Agency Region	Type and number of livestock	Size of upstream catchment (ha)	Soil type	Slope of land (%)	Stream size	Regional rainfall over study period[a]
1	Anglian	120 sows (90 pregnant)	65	Clay	3-7	0.6m wide 0.1m deep	46-74
2	South West	200 dairy cows 200 lambs	74.3	Clay	5-10	1.5m wide 0.08m deep	47-80
3	South West	110 dairy cows (70 pregnant) 140 pregnant ewes	71.6	Silt-clay loam	10-20	1.1m wide 0.06m deep	47-80
4	North West	250 dairy cows (30 pregnant) 300 ewes	56	Clay	1-3	0.6m wide 0.1m deep	61-115
6	North East	27 pregnant beef cows	15	Silt	<1	1m wide 0.15m deep	37-117
8	North West	450 dairy cows 300 ewes	80	Fine/coarse loam	3	0.54m wide 0.08m deep	61-115
9	North West	110 dairy cows 170 ewes	70	Fine loam	2	0.88m wide 0.12m deep	61-115
11	North West	Several hundred dairy cows	110	Fine/coarse loam	2	1.64m wide 0.10m deep	61-115
13	North West	65 dairy cows 200-300 ewes	320	Fine loam	<1	1.72m wide 0.43m deep	61-115
14	Wales	22 beef cows	~450	Sandy silt overlying sandy loam	1-2	2.32m wide 0.06m deep	65-90

[a] as a percentage of the 1961-1990 average

Table 3.4 Potential for excreta to reach watercourse

Farm	Livestock housing	Livestock access to stream	Slurry/manure spreading during study period	Potential for farmyard run-off
1	In sheds	No	Dirty water spread twice a week	High
2	Some cows and all lambs on field	Yes	Manure spread at start of study over 70% farm at 12.5-25 m^3/ha	Low
3	Cows on field for first two weeks of sampling, ewes on field	Yes	Fresh slurry applied to two fields at 22,500 l/ha	High
4	Cows in sheds, ewes on field	Yes	Slurry applied at 28 m^3/ha	Low
6	In sheds	Yes	Slurry applied at 6,300 l/ha and manure applied at 10 tonnes/ha	Low
8	Cows in sheds, ewes on field	Yes	No	No
9	Cows in sheds, ewes on field	Yes	Some manure applied	No
11	In sheds	No	Slurry applied at ~50,000 l/ha before sampling began	Low
13	Cows in sheds, ewes on field	Yes	Slurry applied at ~40,000 l/ha before sampling began	Low
14	On field	Yes	No	No

Information on the hormonal veterinary medicines used on the study farms during the POCIS deployment and for the preceding 12 months is shown in Table 3.5. All farms supplied information, except Farm 9. One active ingredient was reported that did not appear on the list of approved actives supplied by the VMD (Table 2.2). This was flugestone acetate, which was used to treat ewes on Farm 8 and so did not appear on the list of approved actives for cattle and pigs products.

Table 3.5 Hormonal veterinary medicine use on farms November 2003-January 2005

Farm	Date	Trade Name	Active
1	Throughout	Regumate	Altrenogest 15 ml/day in feed[a]
2	12/03	CIDR	Progesterone 1.9 g[a]
2	12/03	Estrumate	Cloprostenol 0.5 mg[a]
2	1/04	CIDR	Progesterone 1.9 g[a]
2	1/04	Estrumate	Cloprostenol 0.5 mg[a]
2	2/04	CIDR	Progesterone 1.9 g[a]
2	2/04	Estrumate	Cloprostenol 0.5 mg[a]
2	4/04		Oxytocin 5 ml[a]
2	11/04		Progesterone[a]
2	12/04	CIDR	Progesterone 1.9 g[a]
2	12/04	Estrumate	Cloprostenol 0.5 mg[a]
3	12/03	Estrumate	Cloprostenol 0.5 mg[a]
3	1/04	Estrumate	Cloprostenol 0.5 mg[a]
3	3/04	Estrumate	Cloprostenol 0.5 mg[a]
3	11/04	Estrumate	Cloprostenol 0.5 mg[a]
4			None used
6			None used
8	11/03	CIDR	Progesterone 3.88 g
8	11/03	Estrumate	Cloprostenol 1 mg
8	11/03	Ovagen	Ovine FSH 21.12 mg
8	11/03	PRID	Progesterone 7.75 g
			Oestradiol benzoate 50 mg
8	11/03	Lutalyse	Dinoprost 225 mg
8	11/03	Receptal	Buserelin 0.04 mg
8	12/03	Estrumate	Cloprostenol 0.5 mg
8	12/03	Ovagen	Ovine FSH 10.56 mg
8	12/03	PRID	Progesterone 7.75 g
			Oestradiol benzoate 50 mg
8	12/03	CIDR	Progesterone 7.76 g
8	12/03	Lutalyse	Dinoprost 225 mg
8	12/03	Receptal	Buserelin 0.02 mg
8	1/04	Pluset	FSH 1425 iu
			LH 1425 iu
8	1/04	PRID	Progesterone 10.85 g
			Oestradiol benzoate 70 mg
8	1/04	CIDR	Progesterone 40.74 g
8	1/04	Lutalyse	Dinoprost 775 mg
8	2/04	Estrumate	Cloprostenol 1.5 mg
8	2/04	PRID	Progesterone 10.85 g
			Oestradiol benzoate 70 mg

8	2/04	CIDR	Progesterone 19.4 g
8	2/04	Lutalyse	Dinoprost 275 mg
8	2/04	Receptal	Buserelin 0.06 mg
8	3/04	CIDR	Progesterone 1.94 g
8	3/04	Estrumate	Cloprostenol 0.5 mg
8	3/04	Pluset	FSH 600 iu
			LH 600 iu
8	3/04	PRID	Progesterone 3.1 g
			Oestradiol benzoate 20 mg
8	3/04	Lutalyse	Dinoprost 300 mg
8	3/04	Receptal	Buserelin 0.02 mg
8	4/04	PRID	Progesterone 1.55 g
			Oestradiol benzoate 10 mg
8	4/04	Lutalyse	Dinoprost 100 mg
8	5/04	CIDR	Progesterone 1.94 g
8	5/04	Estrumate	Cloprostenol 0.5 mg
8	5/04	Pluset	FSH 600 iu
			LH 600 iu
8	5/04	PRID	Progesterone 9.3 g
			Oestradiol benzoate 60 mg
8	5/04	Lutalyse	Dinoprost 375 mg
8	5/04	Receptal	Buserelin 0.02 mg
8	6/04	CIDR	Progesterone 13.58 g
8	6/04	Lutalyse	Dinoprost 150 mg
8	7/04	CIDR	Progesterone 5.82 g
8	7/04	Estrumate	Cloprostenol 1.5 mg
8	7/04	Pluset	FSH 1475 iu
			LH 1475 iu
8	7/04	PRID	Progesterone 18.6 g
			Oestradiol benzoate 120 mg
8	7/04	Lutalyse	Dinoprost 375 mg
8	7/04	Receptal	Buserelin 0.08 mg
8	7/04	Chronogest	Flugestone acetate 180 mg
8	7/04		PMSG 5000 iu
8	8/04	PRID	Progesterone 12.4 g
			Oestradiol benzoate 80 mg
8	8/04	Lutalyse	Dinoprost 237.5 mg
8	8/04	Receptal	Buserelin 0.08 mg
8	9/04	PRID	Progesterone 9.3 g
			Oestradiol benzoate 60 mg
8	9/04	Lutalyse	Dinoprost 137.5 mg
8	9/04	Receptal	Buserelin 0.02 mg
8	9/04	Oxytocin	Oxytocin 4.5 mg
8	9/04		PMSG 5000 iu
8	10/04	Pluset	FSH 500 iu
			LH 500 iu
8	10/04	PRID	Progesterone 13.95 g
			Oestradiol benzoate 90 mg
8	10/04	CIDR	Progesterone 21.34 g
8	10/04	Lutalyse	Dinoprost 550 mg

8	10/04	Receptal	Buserelin 0.04 mg
8	11/04	CIDR	Progesterone 1.94 g
8	11/04	Estrumate	Cloprostenol 0.5 mg
8	11/04	PRID	Progesterone 3.1 g
			Oestradiol benzoate 20 mg
8	11/04	Lutalyse	Dinoprost 50 mg
8	11/04	Oxytocin	Oxytocin 0.9 mg
8	12/04	Pluset	FSH 500 iu
			LH 500 iu
8	12/04	PRID	Progesterone 7.75 g
			Oestradiol benzoate 50 mg
8	12/04	Lutalyse	Dinoprost 275 mg
8	12/04	Receptal	Buserelin 0.04 mg
8	1/05	CIDR	Progesterone 1.94 g
8	1/05	Estrumate	Cloprostenol 0.5 mg
8	1/05	PRID	Progesterone 7.75 g
			Oestradiol benzoate 50 mg
8	1/05	Lutalyse	Dinoprost 175 mg
8	1/05	Receptal	Buserelin 0.04 mg
9			No information supplied
11	11/03	Lutalyse	Dinoprost 25 mg
11	11/03	CIDR	Progesterone 9.7 g
11	11/03	Lutalyse	Dinoprost 650 mg
11	11/03	Receptal	Buserelin 0.16 mg
11	12/03	CIDR	Progesterone 3.88 g
11	12/03	Lutalyse	Dinoprost 275 mg
11	12/03	Receptal	Buserelin 0.14 mg
11	3/04	Lutalyse	Dinoprost 25 mg
11	4/04	Lutalyse	Dinoprost 25 mg
11	7/04	PRID	Progesterone 1.55 g
			Oestradiol benzoate10 mg
11	11/04	Lutalyse	Dinoprost 450 mg
11	12/04	Lutalyse	Dinoprost 675 mg
11	12/04	CIDR	Progesterone 7.76 g
11	12/04	Receptal	Buserelin 0.08 mg
13	11/03	Estrumate	Cloprostenol 0.25 mg/ml[a]
13	12/03	Receptal	Buserelin 0.004 mg/ml[a]
13	1/04	Receptal	Buserelin 0.004 mg/ml[a]
13	2/04	Receptal	Buserelin 0.004 mg/ml[a]
13	3/04	PRID	Progesterone[a]
13	3/04	Estrumate	Cloprostenol 0.25 mg/ml[a]
13	3/04	Receptal	Buserelin 0.004 mg/ml[a]
13	6/04	Oxytocin	Oxytocin[a]
14	2/04		Oxytocin 1 ml[a]

[a] Total dose used on date cannot be calculated as number of treated animals or amount used is not known.

4 Analysis and discussion

4.1 TWA steroid oestrogen concentrations

The results of the steroid oestrogen analyses are shown in Figures 4.1 and 4.2 as TWA concentrations for E1 and E2 respectively. E1 and E2 are natural steroid oestrogens that appear to be common to all vertebrates (Johnson *et al*., 2005).

On six occasions the concentration of E1 was larger at the downstream sampling point than the upstream point (Figure 4.1). Site 4 had the greatest concentration of E1, but unfortunately had no associated upstream sample with which to compare this result. Four sites had higher concentrations of E1 at the downstream sampling point.

E2 was detected on fewer occasions and at lower concentrations than E1 (Figure 4.2). On six occasions the downstream concentration of E2 was greater than the upstream sample concentration. On two occasions (site 9 and 11) the downstream concentration was lower than that of the upstream sample.

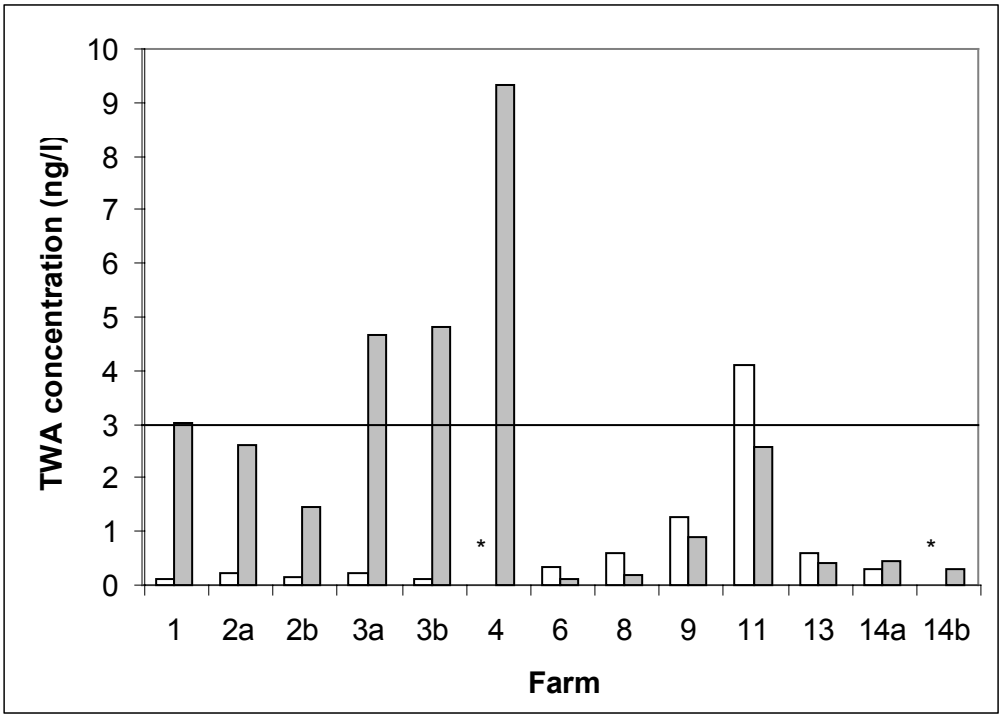
Johnson *et al*. (2005) estimated the amounts of E1 and E2 excreted by various livestock. When combining concentrations in urine and faeces, dairy cattle produced 2.2 times more E1 than E2, pigs 10.4 times more E1, and sheep three times more E1. When pregnant the total amount of oestrogens increased, as did the proportion of E1 (Johnson *et al*., 2005). In addition, E1 has been found to be the primary metabolite of E2 in soil (Das *et al*., 2004) and water (Environment Agency, 2002).

EE2 is a synthetic steroid used in the contraceptive pill. Any EE2 present in the samples could therefore be attributed to a human, rather than agricultural, source. EE2 was not detected at any site.

Environment Agency (2002) derived provisional predicted no-effect concentrations (PNEC) for natural and synthetic steroid oestrogens in surface waters. A PNEC of 0.1 ng/l annual average (AA) was suggested for EE2 and a tentative PNEC of 1.0 ng/l AA for E2. There was insufficient data to derive a PNEC for E1. However, it was noted that relative potency studies suggest that E1 is around three to five times less potent than E2, and therefore a provisional target range of 3.0 to 5.0 ng/l for E1 may be appropriate.

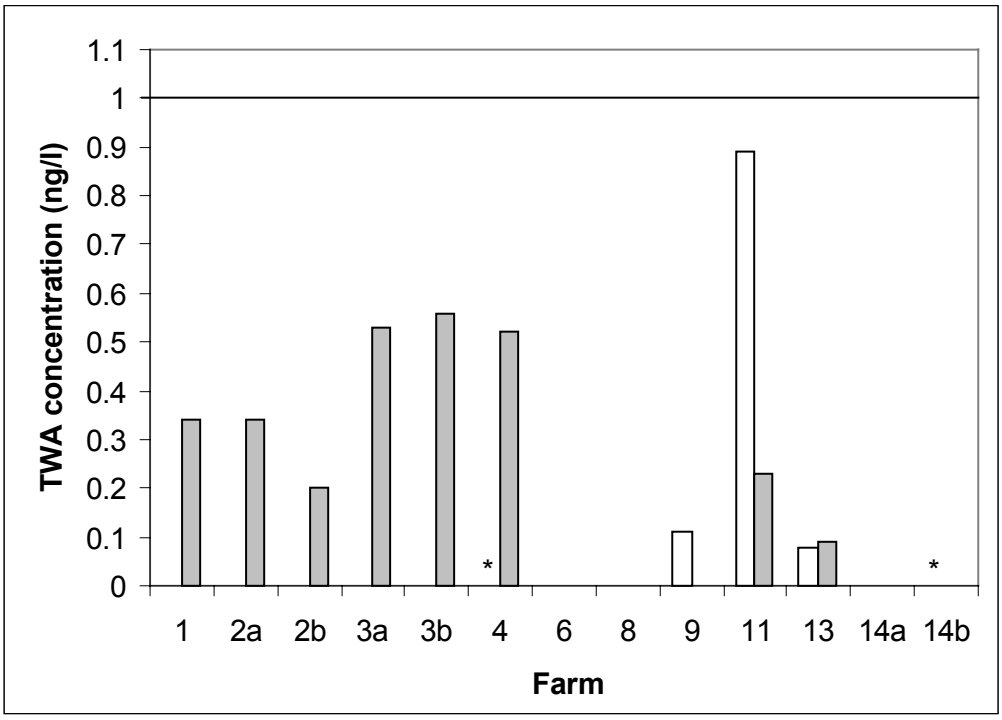
TWA concentrations are the average concentration to which the passive sampler was exposed over the deployment period. They can therefore be compared directly to the AA PNEC. A horizontal line at 3.0 ng/l E1 on Figure 4.1 represents a conservative level below which one would not predict adverse effects on the aquatic community. Five samples exceeded this, although four of these were less than 5.0 ng/l, the upper limit of the provisional target range suggested by Environment Agency (2002). None of the samples exceeded the tentative PNEC for E2 (Figure 4.2).

Figure 4.1 E1 TWA concentration at each site



Upstream sites are white bars, downstream sites grey bars. * shows no data.
Line at 3.0 ng/l represents a conservative level below which one would not expect adverse effects on the aquatic community (Environment Agency, 2002)

Figure 4.2 E2 TWA concentration at each site



Upstream sites are white bars, downstream sites grey bars. * shows no data.
Line at 1.0 ng/l represents the tentative PNEC for E2 (Environment Agency, 2002)

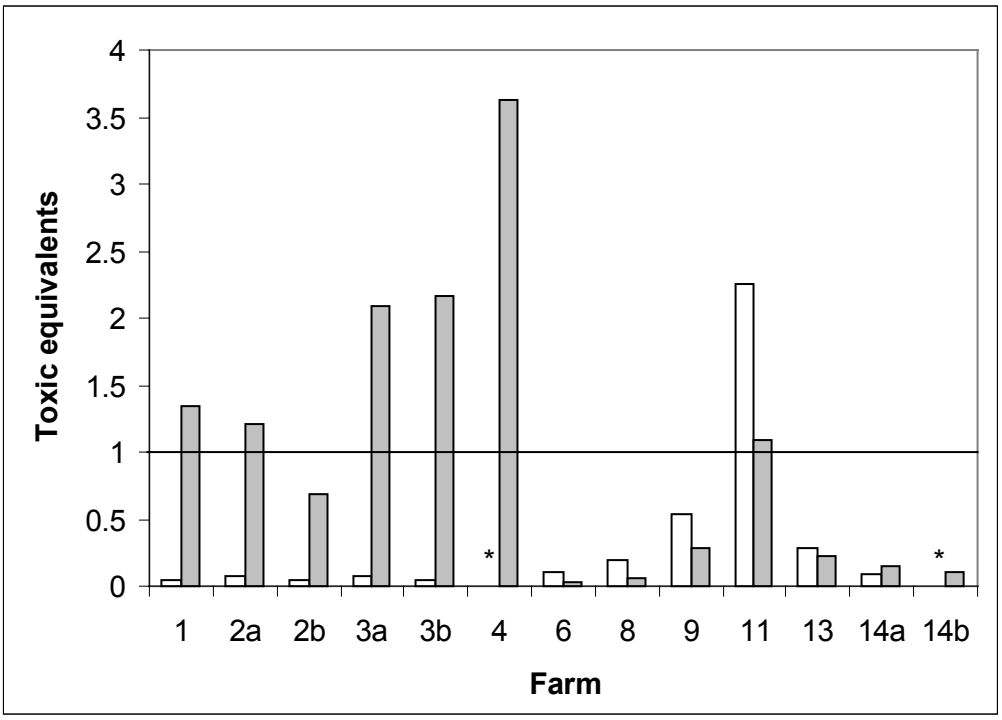
When present in a mixture, the toxicity of steroid oestrogens has been found to be additive (Thorpe *et al*., 2003). The Environment Agency (2002) has suggested a way in which a toxic equivalents approach could be used to determine the potential effect of the total steroid load in the sample. This is calculated as:

$$\frac{[E1]}{PNEC(E1)} + \frac{[E2]}{PNEC(E2)} + \frac{[EE2]}{PNEC(EE2)} = \text{toxic equivalents}$$

At present, detection limits for EE2 make this approach unfeasible, as concentrations below 0.1 ng/l cannot be confidently measured. However, by removing EE2 from the equation and summing the toxic equivalents of E1 and E2, this gives a lower bound to the value. As the PNEC for E2 is 1.0 ng/l, this approach is equivalent to the E2 equivalents calculated in Table 3.2 when a PNEC of 3.0 ng/l is taken for E1.

The toxic equivalents at each site are shown in Figure 4.3. Any values greater than one represent situations where the concentration of total steroids would be of concern. Sites 1 to 4 have toxic equivalents greater than one at the downstream sampling sites. Site 11 has toxic equivalents greater than one at both the upstream and downstream points, although the upstream sampling point has the higher value.

Figure 4.3 Total steroid toxic equivalents at each site



Upstream sites are white bars, downstream sites grey bars. * shows no data. Line at 1.0 toxic equivalent represents a concentration below which you would not expect adverse effects on the aquatic community.

These results suggest that steroid oestrogens are entering the surface water environment between the upstream and downstream sampling points at Sites 1 to 3. All three of these farms spread dirty water, manure or slurry during the sampling period and Farms 1 and 3 were also judged to have a high risk of run-off from the farmyard.

Investigations into the fate of steroid oestrogens in soil have concluded that they are unlikely to be highly mobile due to their large log Koc values (~3-4) and therefore that leaching from soil would be limited, with most transport via run-off (Lee *et al*., 2003). Livestock at Farms 2 and 3 also had direct access to the stream, providing a route by which steroid oestrogens could enter directly into the surface water, bypassing the soil.

The calculated TWA concentrations for E1 and E2, and therefore the calculated toxic equivalents, have several limitations. Calibration data used to estimate an uptake rate was provisional and only generated for E2. Analyte recovery from the POCIS discs used for calibration was low (around 50 per cent) and this may have resulted in an underestimate of the uptake rate, and therefore an overestimate of the true TWA concentration. In this report the uptake rate was also used as a surrogate for the E1 uptake rate. Although Matthiessen *et al*. (2005) argue that uptake rates are likely to be similar for compounds that have similar structure and properties, this assumption is likely to add error to the calculations. Passive sampling uptake rates are altered by environmental variables, particularly temperature and flow rate. For polar compounds, it has been suggested that flow rate plays a particularly important role, as the uptake rate is governed by the rate of diffusion through a boundary layer of water next to the membrane (Alverez *et al*., 2004). No measurements of flow or temperature were taken at the sites and so no adjustment could be made for these variables. Finally, the analytical quantification had an error of up to 50 per cent. These factors suggest that the TWA concentrations calculated here should be seen as estimates only.

Nevertheless, concentrations calculated here are similar to those reported elsewhere. Several studies from outside the UK have investigated the input of steroid oestrogens into surface waters from agricultural activities. However, the majority of these focus on inputs from poultry litter (such as Shore, Correll and Chakraborty, 1995; Nichols *et al*., 1997; Finlay-Moore, Hartel and Cabrera, 2000) and are not discussed further here.

Kolodziej *et al*. (2004) took samples from surface water bodies in agricultural landscapes dominated by dairy farming. E1 was detected in 45 per cent of river samples up to a maximum concentration of 0.9 ng/l and 47 per cent of irrigation canals up to a maximum concentration of 17 ng/l. E2 was also detected, although at fewer sites (9 per cent rivers and 7 per cent irrigation canals) and at lower concentrations (0.6 ng/l and 0.7 ng/l respectively). Shore *et al*. (2004) measured concentrations of up to 6.0 ng/l oestrogen (oestrone plus oestradiol) in a canal receiving run-off from cattle pasture. Irwin, Gray and Orberdorster (2001) reported levels of E2 up to 1.8 ng/l in ponds receiving run-off from beef cattle pasture.

The US Geological Survey sampled 139 streams in 30 different states, focusing on locations that were downstream of intense urbanisation or livestock production (Kolpin *et al*., 2002). E1, E2 and EE2 were analysed for at 70 sites and were detected at up to 10 per cent of these. Concentrations were reported as maximum and median detected concentration: E1 112 ng/l max, 27 ng/l median; E2 93 ng/l max, 9 ng/l median; EE2 273 ng/l maximum, 94 ng/l median. The relatively high concentration of EE2 demonstrates the anthropogenic inputs to the water bodies

sampled, and this may explain the much greater concentrations seen in this study compared with others.

4.2 Hormonal veterinary medicine analysis results

Qualitative data on the types of hormonal veterinary medicines used were collected from nine of the ten farms. Over fifty percent of the active ingredients approved for the treatment of cattle or pigs were used on at least one occasion on the study farms between November 2003 and January 2005. Three of the five veterinary medicines for which analytical methods were developed were used during this period: cloprostenol, dinoprost and progesterone.

Quantitative data on the amount used was only received from four farms, two of which used no hormonal veterinary products during the time period in question. The quantities of the different actives used on these two farms are shown in Table 4.1. In terms of weight, progesterone was by far the most used veterinary hormone, with dinoprost the second most used.

Table 4.1 Total amount of active ingredient used on the farms that suppliedquantitative information for the period November 2003-January 2005

Active ingredient	Total amount used (mg)
Progesterone	267170
Dinoprost	6350
Oestradiol benzoate	810
FSH	236[a]
Flugestone acetate	180
Dexmethasone	96
LH	15[a]
Cloprostenol	6.5
Oxytocin	5.4
Buserelin	0.8
PMSG	10000 iu

[a] Converted from iu to mg

None of the veterinary medicines analysed for were detected at concentrations greater than the limits of detection, which could be explained in a number of ways. The compounds might not be accumulated on the POCIS discs, the extraction process might not work for these compounds, or the compounds may not have been present in the environment at sufficient concentration to be detected. However, as part of the analytical method, standards were run through Oasis sorbant, and then extracted in order to generate calibration curves. This demonstrated that the five compounds for which methods were developed were all picked up by POCIS, and were able to be extracted successfully. This therefore suggests that these compounds were simply not present in the environment.

As noted above, two of the compounds analysed for (carbetocin and oestradiol valerate) were not used at any of the farms during the sampling period, or during the previous year. One would therefore not expect these compounds to be present.

Progesterone was used at four farms (2, 8, 11, 13), cloprostenol four farms (2, 3, 8, 13) and dinoprost two (8, 11). However, Farm 13 did not use any of these compounds in the six months leading up to sampling, or during the sampling period.

Farms 8, 11 and 13 showed no evidence of steroid oestrogen input from the livestock. However, results from the steroid oestrogen analyses suggest that oestrogens are entering the water bodies at Farms 2 and 3. The fact that no veterinary medicines were detected in samples from these two farms may be for several reasons. Concentrations of the veterinary medicines in excreta would be predicted to be much lower than the concentrations of natural steroid oestrogens, and might simply be too low to detect. Alternatively, the pathway from the livestock to the water body might not be present for the veterinary medicines. For example, they might degrade rapidly or sorb strongly to soil/sediments.

5 Conclusions

Once released into the environment, natural steroid oestrogens and hormonal veterinary medicines have the potential to act as endocrine disrupters to non-target organisms. This study aimed to measure the amounts of these substances entering the local aquatic environment from intensive livestock rearing facilities.

Greater concentrations of E1 and E2 were detected at sites downstream of the farms compared to upstream sites at several of the locations studied. This suggests that intensive livestock rearing does contribute E1 and E2 to surface waters at some farms.

Some of the study sites had greater concentrations of steroid oestrogens at the upstream sites. The reason for this is unclear. However, as EE2 was not detected the sources are unlikely to be anthropogenic.

During this study, concentrations of E1 and oestrogenic toxic equivalents were detected above levels of concern. This suggests that these compounds may potentially be exerting an adverse effect on aquatic communities.

None of the veterinary medicines monitored for were detected at concentrations above the limits of detection. Laboratory calibrations suggest that these compounds were not present in the water bodies at sufficient concentrations, rather than the sampling technique being unsuitable.

6 Recommendations

Listed below are the main recommendations generated from this project.

Concentrations of steroid oestrogens were detected at levels where one might predict adverse effects on the aquatic community. Further work could be carried out to determine concentrations at a greater number of sites over a longer time period, to determine whether this is a widespread issue. In addition, work could be carried out to look for impacts on aquatic populations rather than simply chemical concentrations.

The concentration of steroid oestrogens at upstream sites was sometimes greater than concentrations downstream. This suggests there are additional sources to the ones identified in this study. Further work is therefore necessary to identify the sources of oestrogenic activity so that if reduction measures are needed, they can be properly targeted.

POCIS appears to be a suitable way in which to monitor steroid oestrogen concentrations over a period of time. In order to maximise the information gained from monitoring studies, calibration data should be generated so that concentrations on the sampler can be converted back to a TWA concentration with confidence.

The hormonal veterinary medicines monitored for in this study were not detected in the samples from any of the farms. Future work investigating the presence of veterinary medicines in the environment should monitor different media, such as manure/slurry, soil and water. This would give a better indication of whether veterinary medicines are being released into the environment and at what concentrations, along with their environmental fate.

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Glossary of terms

Endocrine disrupting	A substance or mixture of substances that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny or populations.
Endocrine system	A system of glands that secrete hormones into the bloodstream.
Excreta	Faeces and urine.
Hormonal veterinary medicine	A veterinary medicine that is used to regulate certain organs or processes such as oestrus. These compounds are often man-made (synthetic) versions of naturally occurring hormones.
Passive sampler	A monitoring device deployed *in-situ* to sample over a period of time. Samples by diffusion rather than active sampling.
Steroid oestrogen	A group of natural or synthetic female sex hormones, which stimulate the function of the female reproductive organs and may also exert an endocrine disrupting effect.
Time weighted average	Concentration calculated by converting the mass accumulated on the passive sampler to an average environmental concentration over the deployment period.

List of abbreviations

AA	Annual average
E1	Oestrone
E2	17 beta oestradiol
EE2	17 alpha ethinyl oestradiol
FSH	Follicle stimulating hormone
iu	International unit
LCMS/MS	Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
LH	Luteinising hormone
LOD	Limit of detection
PMSG	Pregnant mare serum gonadotrophin
PNEC	Predicted no-effect concentration
POCIS	Polar Organic Chemical Integrative Sampler
TWA	Time weighted average
VMD	Veterinary Medicines Directorate

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