

## 2006 Commentary on non-compliant results

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### 1. National Surveillance Scheme (NSS)

Samples collected under the UK National Plan may be taken at abattoirs or on-farm, and provide retrospective surveillance data. As a consequence, carcasses are not detained pending the laboratory result.

#### a) Prohibited and unauthorised substances

**1. Zeranol.** Zeranol is a growth promoting hormone and taleranol is its principal metabolite. Administration of zeranol and taleranol to food-producing animals is banned in the EU. However, both compounds and other related metabolites can be found naturally in urine from various animal species, if their feed contains zearalenone, a chemical produced naturally by certain fungi. Zeranol and the *Fusarium spp.* toxins were detected in a urine sample taken from one bovine. Statistical analysis developed at VSD indicated that these results were due to metabolism of the naturally-occurring fungal metabolites, and not as a result of zeranol abuse.

**2. Progesterone.** Progesterone is a naturally-occurring female hormone that can also occur at low concentrations in male animals. It is illegal to administer progesterone to food-producing animals to promote growth in the EU. Serum residues of progesterone were found in blood samples from three male bovines in 2006. Where follow-up investigations were possible (some investigations had to be cancelled as a result of increased workloads associated with the findings of nortestosterone in emergency slaughter animals), no evidence of abuse was found. No further action is proposed

**3. Testosterone.** Testosterone is a naturally-occurring male hormone that can also occur at low concentrations in female and castrated animals. It is illegal to administer testosterone to food-producing animals to promote growth in the EU. Serum residues of testosterone were found in a blood sample from one female bovine in 2006. Confirmation of this result was considerably delayed by the increased sample load on the laboratory during the rest of this year. The DARD/FSANI/AFBI Residues Action Group agreed that field investigation would serve no purpose at this time. No further action was proposed.

**4. Nortestosterone.** Nortestosterone is a growth-promoting hormone. It is illegal to administer nortestosterone to food-producing animals in the EU. Residues of  $\alpha$ -nortestosterone, the main metabolite of nortestosterone in cattle and sheep, were detected in urine samples collected from three sheep and one heifer (at a concentration marginally in excess of the VMD Action Limit of 5 ppb) in 2006. In the sheep, a follow-up investigation revealed no evidence of abuse. In the heifer, the animal was resampled and the nortestosterone concentration was below the VMD Action Limit. No further action is proposed.

**5. Phenylbutazone.** Phenylbutazone is a non-steroidal anti-inflammatory painkiller that is not licensed for use in cattle or other food-producing animals (although it is licensed for horses that are excluded from the human food chain). Plasma residues of phenylbutazone were detected in a blood sample taken at slaughter from a healthy two year old beef heifer in 2006. At the farm follow-up visit, the medicines records were found to be up to date. There were no equine medicines or horses on site. The cause of this result remains unknown.

## b) Veterinary medicines

- 1. Nicarbazin.** Nicarbazin is a coccidiostat, licensed for use in poultry. Whilst there is currently no EU Maximum Residue Limit (MRL) for this compound in broiler liver, the UK has adopted an international MRL (200 µg/kg) as an interim measure, pending the establishment of an EU MRL. One sample of broiler liver was found to contain residues of nicarbazin at a concentration of 224 µg/kg. Confirmation of this result was considerably delayed by the increased sample load on the laboratory during the rest of this year. The DARD/FSANI/AFBI Residues Action Group agreed that field investigation of these would serve no purpose at this time. No further action was proposed.
- 2. Oxfendazole.** Oxfendazole is an anthelmintic (wormer). Several preparations of oxfendazole, and its metabolic precursor, fenbendazole, are licensed for use in cattle, sheep and or pigs, but not in chickens. There is no MRL for this species. It was detected at low levels below the MRL for ruminants, pigs and equidae, in 2 chicken liver samples taken at different slaughterhouses. A follow-up investigation reported that at one of the source farms, the sampled free range flock had had access to pasture recently grazed by lambs treated with this medicine. At the other farm, the birds were kept indoors. Both farms were supplied by one feed mill, but the retained feed samples for these flocks were no longer available when the result was reported. The cause of these results remains unclear. These farms were flagged for further sampling at slaughter.

## 2. Additional testing on NSS samples

The analytical method used to detect hormones in the NSS in Northern Ireland is capable of detecting a wide range of hormones in addition to the compound specifically targeted in the NSS. This section details those positive results found as a result of this non-target analysis. It is anticipated that, starting in 2008, the whole of the UK NSS will be carried out using a similar multi-residue method and this category of result will be incorporated into the main NSS results section.

### a) Prohibited and unauthorised substances

- 1. Zeranol.** Zeranol is a growth promoting hormone and taleranol is its principal metabolite. Administration of zeranol and taleranol to food-producing animals is banned in the EU. However, both compounds and other related metabolites can be found naturally in urine from various animal species, if their feed contains zearalenone, a chemical produced naturally by certain fungi. Zeranol/taleranol and the *Fusarium spp.* toxins were detected in urine sample taken from four bovines and two sheep. Statistical analysis developed at VSD (developed for cattle, but applied in this case to the sheep samples as well) indicated that these results were due to metabolism of the naturally-occurring fungal metabolites, and not as a result of zeranol abuse.
- 2. Progesterone.** Progesterone is a naturally-occurring female hormone that can also occur at low concentration in urine taken from male animals. It is illegal to administer progesterone to food-producing animals to promote growth in the EU. Residues of progesterone were found in the urine from two male bovines in 2006, at a concentration exceeding a provisional upper limit of normality set in the laboratory. Follow-up investigations revealed no evidence of abuse and no further action is proposed.
- 3. Testosterone.** Testosterone is a naturally-occurring male hormone. It is illegal to administer testosterone to food-producing animals to promote growth in the EU. Residues of testosterone were detected in urine collected from 7 male bovines at a concentration greater than the laboratory's statistically-based upper limit of normality for steers. Farm follow-up investigations found no evidence of hormone abuse, follow-up samples were compliant, and it was established that at least 3 of the sampled animals had actually been bulls. The farmers concerned were advised to forward accurate livestock details to the APHIS herd database and to abattoirs. Confirmation of a number of results was considerably delayed by the increased sample load on the laboratory during the rest of this year. The DARD/FSANI/AFBI Residues Action Group agreed that field investigation of these would serve no purpose at this time. No further action was proposed.
- 4. Boldenone.** Boldenone is a growth-promoting hormone. It is illegal to administer boldenone to food-producing animals in the EU. However, it is known that boldenone can occur naturally if certain natural chemicals are present in the animals' diets. The EU has set criteria against which to interpret boldenone results.  $\alpha$ -Boldenone was detected in three bovine and one ovine urine samples in 2006. All of these results indicated a natural cause (faecal contamination of the urine samples) and no further action was taken. (The sheep sample also contained a low level of  $\alpha$ -nortestosterone, within the advisory limit for females, but this animal's gender was unrecorded)

5. **Nortestosterone.** Nortestosterone is a growth-promoting hormone. It is illegal to administer nortestosterone to food-producing animals in the EU. Residues of  $\alpha$ -nortestosterone, the main metabolite of nortestosterone in cattle and sheep, were detected in urine samples from 5 male cattle, 8 female cattle (at concentrations in excess of the Action Limit established by VMD of 5.0  $\mu\text{g/L}$ ) and two sheep (gender unknown). Progesterone was also detected in one of the male cattle, at a similar level to when detected without other hormones in steers; it was speculated that this combination might comprise a stress response. In another of the steers, progesterone was detected at a low level, and oestradiol at a high level (similar to levels found in breeding females); this animal retested negative for all three hormones at a farm follow-up visit, and no evidence of abuse was reported. It was speculated that the first result was due to natural hormone fluctuations preceding puberty, as this animal was 5 months old when first sampled. The female cattle were pregnant and nortestosterone is known to occur naturally in pregnant cattle. No evidence of abuse was evident in all of the follow-up investigations that were carried out. In one case, the animal was a direct import from the Irish Republic and the appropriate authorities were notified. No further action is indicated.

6. **Oestradiol.**  $17\alpha$ -Oestradiol is the main bovine metabolite of  $17\beta$ -oestradiol, a growth promoting hormone. It is a naturally-occurring female hormone that can also occur at low levels in males. It is illegal to administer oestradiol to food-producing animals to promote growth in the EU. This hormone is now accepted to be a complete carcinogen, and the EU has banned it also from inclusion in veterinary medicines for food-producing animals (to treat female reproductive conditions). Residues of  $17\alpha$ -oestradiol were detected in urine collected from three male bovines at concentrations in excess of a tentative upper limit of normality established at VSD, in combination with testosterone. No evidence of abuse was detected at follow-up; it was speculated that incomplete castration may have contributed to these results, and no further action is indicated.

### 3. Bovine QA Scheme

Bovine QA samples are taken at abattoirs, and are designed to provide retrospective surveillance data. As a consequence, carcasses are not detained pending the laboratory result.

#### a) Prohibited and unauthorised substances

- 1. Progesterone.** Progesterone is a naturally-occurring female hormone that can also occur at low concentration in urine taken from male animals. It is illegal to administer progesterone to food-producing animals to promote growth in the EU. Residues of progesterone were found in the urine from three male bovines in 2006, at a concentration exceeding the provisional upper limit of normality set in the laboratory. Follow-up investigations revealed no evidence of abuse and no further action is proposed.
- 2. Zeranol.** Zeranol is a growth promoting hormone and taleranol is its principal metabolite. Administration of zeranol and taleranol to food-producing animals is banned in the EU. However, both compounds and other related metabolites can be found naturally in urine from various animal species, if their feed contains zearalenone, a chemical produced naturally by certain fungi. Zeranol/taleranol and the *Fusarium spp.* toxins were detected in urine sample taken from five bovines. Statistical analysis developed at VSD (developed for cattle, but applied in this case to the sheep samples as well) indicated that these results were due to metabolism of the naturally-occurring fungal metabolites, and not as a result of zeranol abuse.
- 3. Nortestosterone.** Nortestosterone is a growth-promoting hormone. It is illegal to administer nortestosterone to food-producing animals in the EU. Residues of  $\alpha$ -nortestosterone, the main metabolite of nortestosterone in cattle was detected in two male cattle. No evidence of abuse was evident in all of the follow-up investigations that were carried out. In one case, the animal was a direct import from the Irish Republic and the appropriate authorities were notified. No further action is indicated.
- 4. Clenbuterol.** Clenbuterol is a beta-agonist vasodilator that is licensed for use in horses as an asthma treatment, and for short term use in parturient cows as a uterine relaxant. It is not licensed for administration to male cattle and is banned for use as a growth promoter in farm animals. It has been used illegally as a growth promoter in cattle, either alone or in combination with corticosteroid hormones. After slaughter, retina (at the back of the eye) may be tested for clenbuterol, as it can be detected there for up to a year after administration (usually in feed or water). In the spring clenbuterol was found in the retina of a steer sampled under the Bovine QA Scheme at slaughter, prompting the collection of a large number of samples at a farm follow-up visit (bovine hair and urine, feed and water). Clenbuterol was detected at low levels in 1 male and 8 female hair samples. These animals were restricted for re-sampling at slaughter, and the farm was flagged for continued testing at slaughter (with carcase detention pending results). Clenbuterol was not detected in over 100 animals from this farm sampled during the remainder of 2006 and into 2007, and sampling was discontinued in August 2007.
- 5. Oestradiol.**  $17\alpha$ -Oestradiol is the main bovine metabolite of  $17\beta$ -oestradiol, a growth promoting hormone. It is a naturally-occurring female hormone that can also occur at low levels in males. It is illegal to administer oestradiol to

food-producing animals to promote growth in the EU. This hormone is now accepted to be a complete carcinogen, and the EU has banned it also from inclusion in veterinary medicines for food-producing animals (to treat female reproductive conditions). Residues of  $17\alpha$ -oestradiol were detected in urine collected from one male bovine at concentrations in excess of a tentative upper limit of normality established at VSD. No evidence of abuse was detected at follow-up, and no further action is indicated.

6. **Testosterone.** Testosterone is a naturally-occurring male hormone. It is illegal to administer testosterone to food-producing animals to promote growth in the EU. Residues of testosterone were detected in urine collected from 9 male bovines at a concentration greater than the laboratory's statistically-based upper limit of normality for steers. Confirmation of these results was considerably delayed by the increased sample load on the laboratory during the rest of this year. The DARD/FSANI/AFBI Residues Action Group agreed that field investigation of these would serve no purpose at this time. No further action was proposed.

## 4. Meat Inspection Scheme

The carcass is detained at sampling, and excluded from the food chain if a non-compliant result is obtained.

### a) Prohibited and unauthorised substances

1. **Progesterone.** Progesterone is a naturally-occurring female hormone that can also occur at low concentration in urine taken from male animals. It is illegal to administer progesterone to food-producing animals to promote growth in the EU. Residues of progesterone were found in the urine from three male bovines in 2006, at a concentration exceeding the provisional upper limit of normality set in the laboratory. These carcasses were not excluded from the food chain. However, since follow-up investigations revealed no evidence of abuse, no further action is proposed.
2. **Nortestosterone.** Nortestosterone is a growth-promoting hormone. It is illegal to administer it to food-producing animals in the EU. However, this hormone is known to occur naturally in female cattle and sheep at some life stages e.g. during late pregnancy, and VMD (the UK Competent Authority) has set an action level of 5 µg/L in female bovine urine. In 2006 its main metabolite, 17 $\alpha$ ,19-nortestosterone was detected in urine samples taken from five female and 119 male bovine urine samples taken under this scheme.

*Female cattle.* 17 $\alpha$ ,19-Nortestosterone was detected above the VMD Action limit in five cows. Farm investigations reported that two of these had calved less than one week before they were sampled. Clenbuterol was also detected in a hair sample from one of these cows (see report below). A third cow had reacted to a TB skin test (see below); follow-up samples at this third farm tested compliant. These animals were not excluded from the food chain. No evidence of abuse was detected and no further action is proposed.

*Male cattle failing Meat Inspection criteria and excluded from the food chain.* 17 $\alpha$ ,19-Nortestosterone was found in urine samples from 3 male cattle. The meat inspection team reported that one of these was emaciated and contained multiple abscesses, another was suffering from chronic pneumonia, and the third was in good bodily condition but had reacted positively to statutory TB testing (intradermal test for bovine tuberculosis). In view of the earlier result for a female TB reactor, and results emerging during 2006 for injured male cattle (below), the Residues Action Group requested urine samples from additional healthy-seeming male bovine TB reactors. Low levels of alpha-nortestosterone were detected at a low prevalence in this cohort.

*Injured male cattle.* 17 $\alpha$ ,19-nortestosterone were detected in urine samples from a total of 116 injured male cattle (102 steers and 14 bulls). In eight samples, the presence of its metabolic precursor 17 $\beta$ ,19-nortestosterone was also confirmed. Most of the sampled cattle were submitted to abattoirs as on-farm emergency slaughtered (OFES) carcasses; the remainder being found to be injured at ante mortem inspection. At the start of these events, the presence of  $\alpha$ -nortestosterone in male cattle was considered to be indicative of abuse: After this anabolic hormone was detected in a urine sample from a male OFES carcass in early 2006, DARD extended the sampling of casualty



carcasses and injured cattle to abattoirs throughout the province, and positive carcasses excluded from the food chain. However, as the hormone can occur naturally in female cattle, samples from injured females were not analysed. The following lines of evidence, gathered during 2006 and into 2007, indicate that this hormone can arise naturally in injured male cattle:

- Despite intense media attention, there was a steady, continuing occurrence of positive findings in injured male cattle or OFES carcasses submitted to abattoirs throughout the year and throughout the province.
- This cohort (severely injured male cattle) is a very small one that had not previously been systematically sampled for hormones.
- Follow-up samples taken at all of the source farms tested negative, and no evidence of hormones administration was discovered at the farms.
- Two male cattle that tested negative when sampled as healthy animals on-farm, subsequently tested positive when submitted to abattoirs as OFES carcasses.
- Urine samples (49) from injured male cattle/carcasses presented to abattoirs were obtained from 3 countries outside the province.  $\alpha$ -Nortestosterone was detected in 19 of these samples.

Footnote. Subsequently, in 2007, these findings were accepted by UK and EU competent authorities. In light of this, DARD discontinued the routine sampling of all injured animals and OFES carcasses at abattoirs for nortestosterone. Future sampling would be at the discretion of the Meat Inspection teams at the abattoirs. DARD made an *ex gratia* payment reimbursing farmers for the loss of carcasses from injured male cattle condemned following the finding this hormone in them.

Further work to investigate causes of the natural occurrence of nortestosterone in cattle and sheep is needed.

## b) Veterinary medicines

1. **Clenbuterol** is a  $\beta$ -agonist vasodilator that is licensed for use in horses as an asthma treatment, and for short term use in parturient cows as a uterine relaxant. It is not licensed for administration to male cattle and is banned for use as a growth promoter in farm animals. It has been used illegally as a growth promoter in cattle, either alone or in combination with corticosteroid hormones. Clenbuterol was found in the hair of a cow sampled under this scheme at slaughter. (Nortestosterone was also detected in a urine sample from this cow). The carcass was condemned, as there was no farm medicines record of treatment with Planipart. Follow-up hair and urine samples taken from this farm tested negative for clenbuterol.
2. **Oxytetracycline.** Oxytetracycline is an antibiotic that is licensed for use in a wide range of animal species. Residues of oxytetracycline, above the MRL, were found in the muscle of five cattle and one sheep. Follow-up investigations reported inadequate medicines recording in 5 of these cases. One bovine and one ovine sample were from animals that were bought shortly before slaughter, and there was no available record of prior treatments. The meat inspection team found injection sites in the bovine carcass and sampled it for drug residues; the case was referred to GB authorities to investigate as this animal had been imported from Great Britain for slaughter. Two bovines from different farms had been treated with this

antibiotic, but these treatments had not been recorded for these animals, which were slaughtered within the products' withdrawal times; one of these farms kept no medicines records. One bovine was found to be lame at *ante mortem* inspection, prompting sampling. At follow-up, farm medicines recording was generally incomplete and there was no record of this animal having been treated. The farmers concerned were advised in writing of their responsibility to record treatments and implement withdrawal times. In the final case, the positive animal had been treated with an appropriate dose of this antibiotic and the withdrawal time was completed before slaughter. However, the drug had not been administered (in divided doses) as directed in the manufacturer's datasheet. It was concluded that an excessive tissue depot, and possibly the animal's poor clinical condition, could have led to the MRL violation.

3. **Marbofloxacin.** Marbofloxacin is an antibiotic that is licensed for a range of animal species, and may be selected to treat conditions arising late in production as it has short withdrawal times. Marbofloxacin was detected above the MRL in one porcine muscle sample. A follow-up investigation reported generally good medicines recording and identification of treated animals (which are normally marked when they are treated); and that an employee may have mistakenly injected a pig with this drug two days before it was slaughtered. This animal could thus have been slaughtered a few hours within the drug's 48 hour withdrawal time. The producer was allocated to (Pigs Scheme) Phase 2 sampling; three sampling rounds screened negative for antibiotics, and no further action is proposed.

## 5. Pigs Testing Scheme

At Phase 1, the carcass is not detained at sampling, but if found to contain non-compliant residues, the producer is allocated to Phase 2 intensified sampling with carcass detention. Non-compliant carcasses at Phase 2 are condemned. (After 3 consecutive, clear rounds of Phase 2 sampling, the producer is returned to Phase 1 sampling).

### a) Prohibited and unauthorised substances

No positive samples were found.

### b) Veterinary medicines

**Sulphadiazine** is an antibiotic that is licensed for use in a range of species, including injectable, in-water and in-feed preparations for pigs. It was detected at levels above the MRL in two Phase 1 kidney samples from different producers. Follow-up investigations reported that both farms kept good medicines records, but the cause of the first result remains unclear, as this farmer does not use this drug, and there were no records of treatment with it. The second result was probably due to the farmer mistakenly applying the shorter, bovine withdrawal time after injecting a pig with a preparation that is licensed for both species. These producers were allocated to Phase 2 sampling, which tested compliant for the first producer who was returned to Phase 1 sampling. Phase 2 samples were not taken this year for the second producer, but further Phase 1 samples from this farm tested compliant.

**Chlortetracycline** is an antibiotic that is licensed for in-feed use in a range of species, including pigs. It was detected at levels above the MRL in two Phase 1 kidney samples from different producers. Follow-up investigations reported that at one farm, medicated weaner diet may have mistakenly been fed at a later stage of production, and at the other, it was speculated that diet cross contamination within an automated feed delivery system could have caused this result. These producers were allocated to Phase 2 sampling, but one farm stopped pig production. Phase 2 samples from the second producer were not received this year, but further Phase 1 samples tested compliant.

## 6. Other sampling in 2006

### **Notifications from the Republic of Ireland**

The Republic of Ireland detected Penicillin G above the MRL in a sow carcass sampled at slaughter in the ROI, and notified DARD. The farm of origin had sold this sow to a dealer who exported it to the ROI for slaughter there. A follow-up investigation reported that the producer had treated this pig with a Penicillin G preparation and it had been slaughtered within the product's withdrawal time. The producer was allocated to (Pigs Scheme) Phase 2 sampling; three rounds tested compliant and this producer was returned to Phase 1 sampling. It was noted that although the farm could individually identify the treated animal and other animals in the consignment (by ear tag and tattoo), the sampled animal's identity was not recorded in the ROI. DARD's Veterinary Service has requested this information for residues notifications, noting that individual pig identification is required at export under the Aujeszky's Disease control order.

### **Phenylbutazone survey**

Phenylbutazone is a non-steroidal anti-inflammatory painkiller that is licensed for use in horses that are excluded from the human food chain. It is not licensed for use in food-producing animals. The Residues Action Group agreed to sample horses slaughtered in Northern Ireland for export to Europe (in addition to National Surveillance Scheme sampling). Due to limited carcass refrigeration capacity at the designated abattoir, it was not possible to detain carcasses pending results. This drug was detected at low levels (< 5 ppb) in 5 of 171 blood samples taken at slaughter. EU Rapid Alerts were issued in respect of the 5 carcasses, so that authorities could if possible trace and exclude them

### **Broiler nicarbazin survey**

The Residues Action Group agreed to survey 200 broiler livers for the coccidiostat (antiprotozoal) poultry feed additive, nicarbazin. 21 of 197 livers received tested above the JECFA advisory limit for this drug. Where available, the corresponding muscle samples to the noncompliant liver samples were also analysed, and tested compliant. Retained samples of finisher and withdrawal diet (unmedicated with this drug) were requested for the 21 flocks. (These diet types are fed in turn to broilers after the nicarbazin-medicated starter and grower diets). Nicarbazin was detected at very low levels (< 1 ppm) in some but not all withdrawal diet samples received. It was detected at similar levels in all but 3 of the finisher diets; in these 3 diets it was detected at slightly higher levels that the laboratory advised could have contributed to the liver results for these flocks. The poultry companies concerned are taking several measures to improve diet changeover and feeding practices, including replacing nicarbazin-medicated feed with another feed at an earlier stage of production, bringing forward the replacement of older feeding systems and staff training.