MILK DRUG RESIDUE SAMPLING SURVEY

Food and Drug Administration
Department of Health and Human Services

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I. EXECUTIVE SUMMARY

The Food and Drug Administration's Center for Veterinary Medicine (CVM) announced a Milk Drug Residue Sampling Survey in November of 2010 with the purpose of determining if dairy farms with a previous tissue residue violation have more drug residues in raw milk than other dairy farms. The sampling survey required the analysis of raw milk samples from individual dairy farms that had been identified as having a previous drug residue violation in tissues from culled dairy cows at slaughter. Raw milk samples were analyzed for antibiotics, non-steroidal anti-inflammatory drugs, and an antihistamine under the sampling survey. These samples were compared to a control group of samples from dairy farms that were not on the list of identified farms with a previous tissue residue violation. As a surveillance-oriented survey, this survey was designed so that samples were double-blinded and it was not possible to trace back samples to any dairy farm, laboratory, or region of the country.

Samples were collected from dairy farms with a previous tissue residue violation (targeted farms) and from a comparable number of randomly selected dairy farms that were not selected for inclusion in the targeted list (non-targeted farms). Samples were tested for 31 different drug residues. A milk sample was considered to be confirmed positive if any one of the 31 drugs with an established tolerance or safe level was found in the sample at a concentration above the tolerance or safe level. If the drug does not have an FDA established tolerance or safe level in milk, samples were considered confirmed positive if the drug residue was above the confirmation of identity. Confirmed positive samples did not meet regulatory standards and would be considered violative if they were identified during regulatory milk testing. Originally, 1918 milk samples were tested. Six samples (0.3% of the samples originally tested) were excluded from the final results and statistical evaluation due to protocol deviations and unresolved discrepancies. Further discussion regarding these excluded samples can be found in the Results section. A total of 15 milk samples (0.78%) were confirmed positive out of the 1912 analyzed (one sample contained two confirmed drug residues). The final results include 11 confirmed positive milk samples out of 953 (1.15%) targeted milk samples representing a total of 12 confirmed positive milk residues in the targeted sample group, including the sample that contained two confirmed drug residues. Out of the 959 non-targeted samples, four of the samples were confirmed positive (0.42%) representing a total of four confirmed drug residues in the non-targeted group.

There was a qualitative difference in the types of confirmed drug residues in each group, with a more varied pattern of confirmed drug residues being found among the targeted population. While florfenicol was found in milk samples from both the targeted dairy farm group and the non-targeted dairy farm group, only samples from the targeted dairy farm group contained additional confirmed drug residues from ciprofloxacin[1], gentamicin, sulfamethazine, tilmicosin, or tulathromycin. All of the six drugs that were found as confirmed residues in this milk survey have also been reported by FSIS as tissue residues found in dairy cows.

None of the confirmed drug residues identified in this milk survey are currently required to be routinely tested for under the Pasteurized Milk Ordinance for Grade "A" milk and milk products. None of the drugs found in the targeted or non-targeted groups are approved by FDA to be administered to lactating dairy cows. This means that FDA has not evaluated the use of these drugs in lactating dairy cattle, including whether milk from treated cows is safe for human consumption.

Although this survey was not designed in a manner to evaluate the overall safety of the United States milk supply, the small number of positives in both the targeted and non-targeted groups is encouraging and the FDA continues to be confident in the safety of the U.S. milk supply.

However, in response to the reported findings, FDA is:

1. continuing to work collaboratively with our State regulatory agency partners and the dairy industry to strengthen the National Conference on Interstate Milk Shipments (NCIMS) drug residue testing program for Grade "A" milk to educate dairy producers on best practices to avoid drug residues in both tissues and milk;

2. utilizing data obtained from this sampling survey to develop FDA's risk ranking for drug residues in milk that will assist NCIMS in modifying Appendix N of the Pasteurized Milk Ordinance (PMO) "Drug Residue Testing and Farm Surveillance" to, as necessary, include testing for more diverse drug classes in milk;

[1] Ciprofloxacin is a human drug and a metabolite of the animal drug enrofloxacin.

3. consulting with State milk regulatory agencies to consider (on a case-by-case basis) collecting milk samples in conjunction with investigating illegal drug residues in tissues involving cull dairy cattle.

II. Background

A. Drug Residues and Food Safety

As farmers work with veterinarians to support the health care of their dairy animals, it is sometimes necessary to treat cows with drugs when they are ill. After a cow is treated with a drug, drug residues may be present in milk or meat if the cow is milked or sent to slaughter before the drug has been metabolized and adequately cleared from its system. In order to help ensure the safety of the human food supply, the United States government regulates both the new animal drug approval process and the allowable concentrations of residues in foods derived from food-producing animals.

New animal drugs are approved by the Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM). As part of the new animal drug approval process, CVM establishes a tolerance, or a concentration that is legally allowed in edible tissues[2]. Tolerances are established to protect human health and are based on a scientific assessment process that each drug must undergo before it is approved for use in food animals. When a new animal drug is approved for use in lactating dairy cows, a tolerance is typically established in milk[3]. Detectable drug residues found in milk at concentrations that are below the established tolerance do not pose a human food safety concern.

CVM also determines slaughter withdrawal periods and milk discard times as part of the new animal drug approval process. A withdrawal period identifies the interval between the last administration of a new animal drug and when the animal can be safely slaughtered for food. A milk discard time applies to female animals that produce milk for human consumption and is the interval between the last administration of a new animal drug and when the milk produced by the animal can be safely consumed by humans.

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[2] 21 CFR Sec 556 Tolerances for Residues of New Animal Drugs in Food: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=556

[3] M-I-05: Tolerance and/or Safe Levels of Animal Drug Residues in Milk: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/ucm077350.htm

B. Drug Residue Testing in Milk

In the United States, drug residues are regulated by both Federal and State food safety programs. One example of this is a Federal/State Cooperative Program, the National Conference on Interstate Milk Shipments (NCIMS). This voluntary coalition was established under the Public Health Service Act to ensure the safety and wholesomeness of Grade "A" milk and milk products in the United States. The FDA and the States participate with industry in NCIMS. FDA publishes the *Grade "A" Pasteurized Milk Ordinance* (PMO)[4] as a model ordinance for States to adopt. To participate in the NCIMS Grade "A" program, regulations in each state must be consistent with the PMO and at least as stringent. Grade "A" milk and milk products that are introduced into interstate commerce must be processed in a Grade "A" facility under the specifications outlined in the PMO and the NCIMS Grade "A" Program.

FDA and state regulatory officials have worked with industry through NCIMS to maintain high standards to ensure the safety of the United States milk supply. Under the NCIMS Grade "A" program, State regulatory agencies are required to report milk testing activities to the National Milk Drug Residue Data Base (NMDRDB). More than 3.7 million tests were reported in the NMDRDB in 2012, the same year CVM's Milk Drug Residue Sampling Survey was conducted[5]. Any milk found to contain illegal drug residues under the NCIMS Grade "A" program is not allowed to enter the human food supply.

The PMO requires that a milk sample be obtained from every bulk tank of raw milk collected at each farm, along with a sample obtained from every truckload of raw milk arriving at a dairy plant. The sample from every arriving truckload of raw milk must be tested for the presence of at least four of six specific Beta-lactam drugs (penicillin, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur). Positive test results lead to the mandatory testing of raw milk samples from each farm which supplied raw milk for that truckload. Beta-lactams are considered the most common class of antibiotics used on dairy farms. However, there are a number of other classes of drugs that are approved and may be used on dairy farms that are not currently required to be routinely tested for in raw milk under the PMO.

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[4] Grade "A" *Pasteurized Milk Ordinance* 2011 Revision: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM291757.pdf

[5] NATIONAL MILK DRUG RESIDUE DATA BASE FISCAL YEAR 2013 ANNUAL REPORT: http://www.kandc-sbcc.com/nmdrd/fy-12.pdf

C. Federal Regulation of Drug Residues in Meat

Drug residues in animals slaughtered for human consumption, often referred to as "tissue residues", are under federal regulation of both the United States Department of Agriculture (USDA), Food Safety Inspection Service (FSIS) and FDA CVM. Under the National Residue Program, USDA FSIS conducts tests on animal tissues to determine the presence of drug residues and reports residues above FDA established tolerances or safe levels to CVM through the Residue Violation Information System.

Drug residue violations in tissues are investigated under FDA's Compliance Program 7371.006: **Illegal Residues in Meat, Poultry, Aquacultured Seafood, and Other Animal Derived Foods[6].** Based on investigations conducted by FDA, drug residue violations involving tissue samples from culled dairy cows have been associated with inadequate farm management practices such as a failure to maintain a valid veterinarian-client-patient-relationship, inadequate treatment records, failure to identify and withhold treated cows from slaughter, or not following labeled directions including: withdrawal periods, dosage, duration of treatment, and route of administration.

D. CVM Milk Drug Residue Sampling Survey

Tissue residue violations in culled dairy cows occur in a small component of an otherwise compliant industry. USDA reports that approximately 3.1 million dairy cattle were slaughtered in the United States in 2012.[7] That year, there were 360 dairy cows with reported violative tissue residues, representing approximately 0.01% of total dairy cows slaughtered.[8] CVM wanted to determine whether farms responsible for tissue residue violations in this small subset of the dairy industry may also cause violative drug residues to be present in raw milk, especially from non-Beta-lactam drugs that are not part of routine testing under the PMO. CVM announced a Milk Drug Residue Sampling Survey in November of

[6] FDA Center for Veterinary Medicine: Illegal Residues in Meat, Poultry, Aquacultured Seafood, and Other Animal Derived Foods: http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/ComplianceEnforcement/ucm112583.htm

[7] USDA 2012 Livestock Slaughter Report: http://usda.mannlib.cornell.edu/usda/nass/LiveSlauSu//2010s/2013/LiveSlauSu-04-22-2013.pdf

[8] United States Food and Drug Administration, Residue Violation Information System (FDA RVIS) Database.

2010 with the primary purpose of determining if dairy farms with a previous tissue residue violation have more drug residues in raw milk than other dairy farms.

Efforts such as this survey and an already existing NCIMS milk testing program help to ensure that any problems that may exist are identified and mitigated through education, voluntary action by the dairy industry, and enforcement, as may be appropriate. When originally announced in 2010, CVM received feedback from State regulators, dairy industry associations, and other affected stakeholders about the survey's potential impact on the milk supply. Concerns were expressed that given the limited availability of validated rapid residue tests for use in milk, dairy farmers would be forced to dump large amounts of milk while waiting for lengthy tests to be completed. In response to the feedback received, CVM developed a sampling survey utilizing a third-party to double blind the origin of the samples. Although the resulting sampling survey was not designed for enforcement or compliance action, it provided an effective approach for collecting the necessary data while providing minimal disruption to the milk supply.

The sampling survey required the analysis of raw milk samples from individual dairy farms that had been identified as having a previous drug residue violation in tissues from culled dairy cows at slaughter. These samples were compared to a control group of samples from dairy farms that were not on the list of identified farms with a previous tissue residue violation. This report provides the results of the CVM Milk Drug Residue Sampling Survey. For the purposes of this report, any drug residues in raw milk that exceeded any FDA-established tolerances or safe levels are reported as "confirmed drug residues." For those drugs for which tolerances or safe levels do not currently exist, positive samples are reported as "confirmed drug residues" when they meet the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues"[9]. Confirmed positive samples did not meet regulatory standards and would be considered violative if they were identified during regulatory milk testing.

[9] CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues": http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf

III. Methodology

A. Sampling plan

Samples were collected from dairy farms with a previous tissue residue violation (targeted farms) and from a comparable number of randomly selected dairy farms that were not selected for inclusion in the targeted list (non-targeted farms). The plan called for the sampling to be conducted utilizing the "Universal Sampling System" as defined under the PMO. This "Universal Sampling System" requires that a raw milk sample is collected at each dairy farm every time milk is picked up at the dairy farm by a trained bulk milk hauler/sampler that has been evaluated by a State regulatory official. This system permits the regulatory agency, at its discretion, at any given time and without notification to the industry, to analyze samples collected by the bulk milk hauler/sampler. The sample is representative of the dairy farm's milking production for the day of collection.

Raw milk samples were analyzed for antibiotics, non-steroidal anti-inflammatory drugs, and an antihistamine under the sampling survey (see Table 1). Furthermore, the survey was double-blinded and each milk sample was identified via the use of an alphanumeric system which designated targeted farms (identified as having a previous drug residue) or non-targeted farms (not identified as having a previous drug residue). As a surveillance-oriented survey, this survey was designed so that it was not possible to trace back samples to any dairy farm, laboratory, or region of the country.

For more information regarding the sample collection procedures, see Appendix A.

Table 1: List of Drug Residues Analyzed in Milk Survey

DRUG	TOLERANCE/SAFE LEVEL (ppb)
Ampicillin (AMP)	10
Cephapirin (CEPH)	20
Cloxacillin (CLOX)	10
Penicillin G (PEN G)	5*
Erythromycin (ERY)	50*
Tylosin (TYL)	50
Ciprofloxacin (CIP) †	0 ‡
Sarafloxacin (SAR)	0 ‡
Chlortetracycline (CTC)	300§
Oxytetracycline (OTC)	300§

DRUG	**TOLERANCE/SAFE LEVEL (ppb)**
Tetracycline (TC)	300§
Doxycycline (DC)	0 ‡
Sulfachloropyridazine (SCP)	10*
Sulfadiazine (SDZ)	10*
Sulfamerazine (SMR)	10*
Sulfadimethoxine (SDM)	10
Sulfamethazine (SMZ)	10*
Sulfapyridine (SPD)	10*
Suflaquinoxaline (SQX)	10*
Sulfathiazole (STZ)	10*
Tripelennamine (TRIP)	20
Thiabendazole (THBZ)	50
5-hydroxyflunixin (FLU-OH)	2
Bacitracin (BAC)	500
Virginiamycin (VIR)	0 ‡
Tilmicosin (TIL)	0 ‡
Florfenicol (FF)	0 ‡
Chloramphenicol (CAP)	0 ‡
Tulathromycin (TUL)	0 ‡
Gentamicin (GEN)	30*
Neomycin (NEO)	150

* Amount listed is a “safe level” from M-I-05-5; an FDA/CFSAN Memorandum of Information Milk Safety Reference dated September 27, 2005.

† Ciprofloxacin is a human drug and a metabolite of the animal drug enrofloxacin.

‡ No tolerance was established for this drug in milk.

§ This tolerance includes both the sum and the individual residues of chlortetracycline, oxytetracycline and tetracycline. The sum of the tetracyclines present should not exceed 300 ppb.

B. Analytical Methods

1. Screening and Confirmation

Sample analysis was performed using a multi-class multi-residue LC-MS/MS method as described in LIB# 4443: "Optimization and Validation of Multi-class, Multi-residue LC-MS/MS Screening and Confirmation Method for Drug Residues in Milk" with memorandum of analysis to include chloramphenicol, florfenicol, and tulathromycin. To accommodate for additional analytes and varying laboratory equipment, the method was modified, optimized, and validated by each laboratory according to FDA protocols.

- LIB# 4443:

  http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM239311.pdf

2. Quantitative follow-up methods

For drugs with an FDA established tolerances for residues in milk, presumptive positive results from the screening methods were also analyzed by quantitative methods to determine the concentration of drug residue(s) present. Presumptive positive is defined as residue being at or above 50 percent of the established safe level/tolerance.

Drugs with no established tolerance in milk were not quantified because no amount is allowed in the milk and this method (LIB 4443) is confirmatory as to the identification of the drug. For those drugs for which tolerances or safe levels do not currently exist, positive residues were defined as those meeting the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues":

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf

IV. Results

Originally, 1918 milk samples were tested for 31 different drug residues. A milk sample was considered to be confirmed positive if any one of the 31 drugs was found in the sample at a concentration above the tolerance or safe level or if the drug does not have a tolerance in milk and was above the confirmation of identity. For a complete data table see http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/ComplianceEnforcement/UCM426354.xlsx

After excluding six samples due to protocol deviations and unresolved discrepancies (see Appendix B for further details), the final results were 16 confirmed positive drug residues in a total of 15 milk samples out of 1912 total samples (one sample contained two confirmed drug residues). The final results include 11 confirmed positive milk samples out of 953 targeted milk samples that represent a total of 12 confirmed drug residues in the targeted sample group. Out of the 959 non-targeted samples, four of the samples were confirmed positive representing a total of four confirmed drug residues in the non-targeted group.

Table 2 displays the number of "confirmed drug residues" as defined by the milk sampling survey. "Confirmed drug residues" include any drug residues in raw milk that exceeded any established tolerances or safe level. For those drugs for which tolerances or safe levels do not currently exist, positive samples are reported as "confirmed drug residues" when they meet the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues"[10]

[10] http://www.fda.gov/downloads/animalveterinary/guidancecomplianceenforcement/guidanceforindustry/ucm052658.pdf

Table 2: Number of Confirmed Drug Residues by Group for Each Drug Residue Tested

Drug Residue Tested	Targeted[£] Group: Number of Confirmed Drug Residues[¶] (953 samples analyzed)	Non-Targeted[µ] Group: Number of Confirmed Drug Residues (959 samples analyzed)	Tolerance/Safe Level (in ppb)
Ampicillin (AMP)	0	0	10
Cephapirin (CEPH)	0	0	20
Cloxacillin (CLOX)	0	0	10
Penicillin G (PEN G)	0	0	5*
Erythromycin (ERY)	0	0	50*
Tylosin (TYL)	0	0	50
Ciprofloxacin (CIP) †	1	0	0‡
Sarafloxacin (SAR)	0	0	0‡
Chlortetracycline (CTC)	0	0	300§
Oxytetracycline (OTC)	0	0	300§
Tetracycline (TC)	0	0	300§
Doxycycline (DC)	0	0	0‡
Sulfachloropyridazine (SCP)	0	0	10*
Sulfadiazine (SDZ)	0	0	10*
Sulfamerazine (SMR)	0	0	10*
Sulfadimethoxine (SDM)	0	0	10

Drug Residue Tested	Targeted[£] Group: Number of Confirmed Drug Residues[¶] (953 samples analyzed)	Non-Targeted[µ] Group: Number of Confirmed Drug Residues (959 samples analyzed)	Tolerance/Safe Level (in ppb)
Sulfamethazine (SMZ)	1	0	10*
Sulfapyridine (SPD)	0	0	10*
Sulfathiazole (STZ)	0	0	10*
Tripelennamine (TRIP)	0	0	20
Thiabendazole (THBZ)	0	0	50
5-hydroxyflunixin (FLU-OH)	0	0	2
Bacitracin (BAC)	0	0	500
Virginiamycin (VIR)	0	0	0‡
Tilmicosin (TIL)	1	0	0‡
Florfenicol (FF)	6	4	0‡
Chloramphenicol (CAP)	0	0	0‡
Tulathromycin (TUL)	2	0	0‡
Gentamicin (GEN)	1	0	30*
Neomycin (NEO)	0	0	150
Totals	**12[β]**	**4**	**--**

¶ Confirmed drug residues are any drug residues in raw milk that exceeded FDA established tolerances or safe levels. For those drugs for which tolerances or safe levels do not currently exist, "confirmed drug residues" have met the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues".

£ Targeted farms are dairy farms that were identified to have a previous tissue residue violation.

µ Non-targeted farms are randomly selected dairy farms that were not selected for inclusion in the targeted list

* Amount listed is a "safe level" from M-I-05-5; an FDA/CFSAN Memorandum of Information Milk Safety Reference dated September 27, 2005.

† Ciprofloxacin is a human drug and a metabolite of the animal drug enrofloxacin.

‡ No tolerance was established for this drug in milk.

§ This tolerance includes both the sum and the individual residues of chlortetracycline, oxytetracycline and tetracycline. The sum of the tetracyclines present should not exceed 300 ppb.

β One targeted sample was identified to contain two confirmed drug residues (Florfenicol, and Tilmicosin). Therefore, in the targeted group there were a total of 12 positive residues but only 11 positive samples.

Qualitative differences between the two groups are observed in Table 2 and Table 3. While florfenicol was found in milk samples from both the targeted dairy farm group and the non-targeted dairy farm group, only samples from the targeted dairy farm group contained additional confirmed drug residues from ciprofloxacin, gentamicin, sulfamethazine, tilmicosin, or tulathromycin.

Table 3: Description of Individual Confirmed Drug Residues in Raw Milk Samples by Drug

Confirmed Drug Residue[¶]	Sample Number	Group (Targeted[£] or Non-targeted[µ])	Results (in ppb)	Tolerance/Safe Level (in ppb)
Ciprofloxacin*	DEN 225	Targeted	Detected†	0
Florfenicol	DEN 096	Targeted	Detected†	0
Florfenicol	DEN 116	Targeted	Detected†	0
Florfenicol	DEN 326	Targeted	Detected†	0
Florfenicol	DEN 525	Targeted	Detected†	0
Florfenicol	DEN 609	Targeted	Detected†	0
Florfenicol	ARL 171	Targeted	Detected†	0
Florfenicol	ARL 216	Non-Targeted	Detected†	0
Florfenicol	ARL 276	Non-Targeted	Detected†	0
Florfenicol	SRL 115	Non-Targeted	Detected†	0
Florfenicol	SRL 313	Non-Targeted	Detected†	0
Gentamicin	DEN 588	Targeted	322	30‡
Sulfamethazine	DEN 109	Targeted	175	10‡

Confirmed Drug Residue¶	Sample Number	Group (Targeted£ or Non-targeted$^{\mu}$)	Results (in ppb)	Tolerance/Safe Level (in ppb)
Tilmicosin§	ARL 216	Targeted	Detected†	0
Tulathromycin	DEN 190	Targeted	Detected†	0
Tulathromycin	DEN 463	Targeted	Detected†	0

¶ Confirmed drug residues are any drug residues in raw milk that exceeded any established tolerances or safe levels. For those drugs for which tolerances or safe levels do not currently exist, "confirmed drug residues" have met the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues".
£ Targeted farms are dairy farms that were identified to have a previous tissue residue violation.
µ Non-targeted farms are randomly selected dairy farms that were not selected for inclusion in the targeted list.
* Ciprofloxacin is a human drug and a metabolite of the animal drug enrofloxacin.
†Detected = Residues that do not have a Safe level or Tolerance and meet the criteria for confirmation of identity as described in Guidance for Industry #118, "Mass Spectrometry for Confirmation of Identity of Animal Drug Residues."
http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf.
‡Tolerances have not been established for any of the drug residues found in this survey. Safe levels for Gentamicin and Sulfamethazine are published in M-I-05-5. See "Tolerance and/or Safe Levels of Animal Drug Residues in Milk":
http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/ucm077350.htm.
For more information about tolerances for residues of new animal drugs in foods, refer to 21 CFR 556.
§ One targeted sample was identified to contain two confirmed drug residues (Florfenicol, and Tilmicosin). Therefore, in the targeted group there were a total of 12 positive residues but only 11 positive samples.

Table 4 displays the numbers of milk samples tested, the numbers and percent of milk samples in which confirmed drug residues were found, and the numbers and percent of milk samples in which drug residues were not found. The majority of samples (over 99%) analyzed during this survey did not contain any confirmed drug residues. A total of 15 milk samples (0.78%) were confirmed positive out of the 1912 analyzed (one sample contained two confirmed drug residues).

The final results include 11 confirmed positive milk samples out of 953 targeted milk samples that represent a total of 12 confirmed drug residues in the targeted sample group. Out of the 959 non-targeted samples, four of the samples were confirmed positive representing a total of four confirmed drug residues in the non-targeted group. The percent of positive samples in the targeted dairy farm population is 1.15% while that in the non-targeted sampled dairy farm population is 0.42%.

The proportions of positive samples in the two study groups were compared by means of a Chi-square test using SAS®. The p-value for this study was 0.0677 which is not statistically significant at a level of 0.05. The relative risk of having a positive sample from a dairy farm in the targeted population as compared to the risk of having a positive sample from a dairy farm in the non-targeted population is estimated to be 2.7880 (95% confidence interval = 0.8846 to 8.7865). Since the 95% confidence interval includes 1 these results do not provide conclusive evidence whether there is or is not an increased risk of violative residues in dairy farms from the targeted group. The confidence interval for this study is consistent with the p-value for the Chi-square test.

Table 4: Numbers of Raw Milk Samples Tested and Numbers (percentages) of Raw Milk Samples with Confirmed Drug Residues* by Study Group

Study Group	Number (Percent) of Samples with At Least One Confirmed Drug Residue¶	Number (Percent) of Samples without any Confirmed Drug Residues¶	Total Number of Samples Analyzed
Targeted£	**11 (1.15%)**	**942 (98.85%)**	953
Non-targeted µ	**4 (0.42%)**	**955 (99.58%)**	959
Total	15 (0.78%)	1897 (99.22%)	1912

¶ Confirmed drug residues are any drug residues in raw milk that exceeded any established tolerances or safe levels. For those drugs for which tolerances or safe levels do not currently exist, "confirmed drug residues" have met the criteria for confirmation of identity as defined in CVM Guidance for Industry 118 "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues".
£ Targeted farms are dairy farms that were identified to have a previous tissue residue violation.
µ Non-targeted farms are randomly selected dairy farms that were not selected for inclusion in the targeted list

V. Discussion

The purpose of the raw milk sampling study was to determine if dairy farms with a previous tissue residue violation have more drug residues in raw milk than other dairy farms. This survey evaluated targeted milk samples from a small subset of the dairy farm population that had been identified to previously have a drug residue in dairy cattle tissues and compared them to a control group of samples from non-targeted dairy farms not on the list identified to have a previous tissue residue violation.

The findings of the CVM's Milk Sampling Survey reveal a small number of overall confirmed positive drug residues in both the targeted (12) and non-targeted groups (4). A total of fifteen confirmed positive milk samples were identified out of the 1912 total samples analyzed (one sample contained two confirmed drug residues). Although this survey was not designed in a manner to evaluate the overall safety of the United States milk supply, the small number of positives in both the targeted and non-targeted groups is encouraging and the FDA continues to be confident in the safety of the U.S. milk supply.

The final results include 11 confirmed positive milk samples out of 953 (1.15%) targeted milk samples representing a total of 12 confirmed positive milk residues in the targeted sample group (one sample contained two confirmed drug residues). Out of the 959 non-targeted samples, four of the samples were confirmed positive (0.42%) representing a total of four confirmed drug residues in the non-targeted group.

There was a qualitative difference in the types of confirmed drug residues represented in each group with a more varied pattern of confirmed drug residues being found among the positive samples from the targeted population. Only one drug, florfenicol, was found in the non-targeted group but the targeted group had six different confirmed drug residues (ciprofloxacin[11], florfenicol, gentamicin, sulfamethazine, tilmicosin, and tulathromycin). None of the drugs found in the targeted or non-targeted groups are approved to be administered to lactating dairy cows. This means that FDA has not evaluated the use of these drugs in lactating dairy cattle, including whether milk from treated cows is safe for human consumption.

[11] Ciprofloxacin is a human drug and a metabolite of the animal drug enrofloxacin.

Certain drugs that are not approved for use in lactating dairy cattle may be used in an extralabel manner,[12] and conditions for legal extralabel use are outlined in 21 CFR 530.20. These conditions include a valid veterinarian-client-patient relationship as well as appropriate measures to assure that no illegal drug residues occur in any food-producing animal subjected to extralabel treatment. However, extralabel use is not allowed in certain drugs and extralabel use cannot result in an illegal drug residue in food for human consumption. Drugs prohibited from extralabel use are listed in 21 CFR 530.41. Two of the drugs detected in this survey (ciprofloxacin and sulfamethazine) are not currently approved for use in dairy cows and are prohibited from extralabel use under 21 CFR 530.41.

The drug ciprofloxacin is a fluoroquinolone that is only approved for human use and is not available in a formulation that is commonly given to cattle. Ciprofloxacin is also the marker residue for enrofloxacin, a fluoroquinolone approved for use in beef and non-lactating dairy cattle. Extralabel use of any fluoroquinolone (including ciprofloxacin and enrofloxacin) is prohibited in food-producing animals. Sulfamethazine is a sulfonamide and is not included in the three sulfonamides (sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine) that are allowed to be administered to lactating dairy cows as label directions indicate, but not in an extralabel manner. The presence of ciprofloxacin and sulfamethazine as confirmed drug residues in raw milk suggests illegal extralabel use of a fluoroquinolone (enrofloxacin or ciprofloxacin) and sulfamethazine, respectively.

The other confirmed drug residues (florfenicol, gentamicin, tilmicosin, tulathromycin) are not prohibited from extralabel use in dairy cows; however, the presence of these confirmed drug residues is illegal and violates the conditions for legal extralabel drug use. In 21 CFR 530 there is an obligation by the veterinarian to "establish a substantially extended withdrawal period prior to marketing of milk, meat, eggs, or other edible products supported by appropriate scientific information" in order to ensure that residues are not present when a drug is used in food producing animals in an extralabel manner.

Study design constraints, including blinded samples, did not allow evaluation of possible causal factors for confirmed drug residues such as specific animal drug practices. However, the qualitative difference in the drugs that were represented in each group is suggestive that different animal drug

[12] PART 530 EXTRALABEL DRUG USE IN ANIMALS
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=530.20

administration or residue avoidance practices may exist between the targeted and non-targeted groups. All of the six drugs that were found as confirmed residues in this milk survey have also been reported by FSIS as violative tissue residues found in dairy cows.[13] Based on investigations of farms with tissue residues in dairy cows conducted by FDA, possible causes of confirmed drug residues in milk include: inadequate farm management practices such as a failure to maintain a valid veterinarian-client-patient-relationship, inadequate treatment records, failure to identify and withhold treated cows from slaughter, or not following labeled directions including: discard times, dosage, duration of treatment, and route of administration[14].

The PMO currently only requires bulk milk pickup tankers to be tested for the presence of at least four of six specific Beta-lactam drugs (penicillin, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur); none of the confirmed non-Beta lactam drug residues identified in this milk survey are currently required to be routinely tested for under the PMO for Grade "A" milk and milk products.

Although the small number of positive drug residues is encouraging, the fact that residues of a variety of non-Beta lactam drugs were detected affirms the importance of ongoing efforts to further strengthen existing milk safety safeguards.

In response to these findings, FDA is:

1. Continuing to work collaboratively with our State regulatory agency partners and the dairy industry to strengthen the NCIMS drug residue testing program for Grade "A" milk to educate dairy producers on best practices to avoid drug residues in both tissues and milk;

2. utilizing data obtained from this survey to develop FDA's risk ranking for drug residues in milk that will assist NCIMS in modifying Appendix N of the PMO "Drug Residue Testing and Farm Surveillance" to, as necessary, include testing for more diverse drug classes in milk; and

3. consulting with State milk regulatory agencies to consider (on a case-by-case basis) collecting milk samples in conjunction with investigating illegal drug residues in tissue involving cull dairy cattle.

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[14] FSIS Data Warehouse(DW), and FSIS Public Health Information System(PHIS)

Appendices

A. Sample Collection Procedures

Sampling was conducted as follows:

1. FDA District Offices were given a confidential list of dairy farms that were identified through a relative risk ranking process as having a previous tissue residue violation (targeted list) and a list of laboratories, identified by State Milk Regulatory Agencies, who receive and hold Universal Samples.

   In addition, FDA Investigators in cooperation with FDA Regional Milk Specialists contacted laboratories and requested a list of dairy farms for which they hold Universal Samples to help expedite sample collection. Access to the list of dairy farms handled by individual laboratories enabled FDA Investigators to identify likely location of samples on their targeted list.

   FDA acknowledged that such lists of dairy farms are considered commercial confidential information and FDA personnel handled this information accordingly. FDA Investigators referenced such lists only for the purpose of identifying the likely location of the samples on the Targeted list.

2. FDA Investigators contacted laboratories in advance to set up a convenient time for sample collection to minimize disruption to the lab. FDA Investigators visited the laboratories identified by State Milk Regulatory Agencies and collected samples from the targeted list and a comparable number of samples from non-targeted farms. The goal was to collect a total of 900 targeted samples and 900 non-targeted samples over the course of the sampling survey. The samples were blinded to ensure that the identity of the samples could not be traced back to a region of the country, State, laboratory, or dairy farm of origin. When collecting samples the FDA Investigators:
   - Referred to a confidential list of targeted farms and requested a specified number of samples.
     - If milk samples from certain dairy farms were not available or if the sample volume was insufficient (less than 1.5 oz.), the Investigator was directed to request alternate samples from the targeted farm list to meet the targeted number of samples to be collected at that visit.

- Requested that the laboratory provide a comparable number of samples from non-targeted farms.
    - FDA Investigator asked the laboratory to provide samples that could serve as samples from non-targeted farms. Laboratory personnel selected the samples from non-targeted farms without any input from the FDA Investigator as to State of origin, farm name, etc.
    - FDA Investigator verified that the proposed non-targeted farms were not included on the confidential list of targeted farms.
    - Information regarding the identity of the non-targeted farms for which samples were collected was not recorded.

3. Samples were then frozen and shipped on dry ice to the Institute for Food Safety and Health (IFSH) at the Illinois Institute of Technology where they were stored at −20°F or below. IFSH received samples from all five FDA regions and routinely held samples for a 2-week period to assure a sufficient pool of samples for randomization. IFSH only recorded the date of receipt of the samples in order to ensure that samples were held for the 2-week period. Other information regarding the samples was not recorded or retained.

4. IFSH then randomly selected from the pool of stored, frozen milk samples and shipped the appropriate number of samples from non-targeted and targeted farms on dry ice to the appropriate FDA laboratory.

5. FDA completed sample collection and analysis within twelve (12) months. FDA collected samples in the most efficient and effective manner, taking into consideration sample storage time and FDA laboratory analytical capability. FDA started sampling in January 2012.
6. All samples were analyzed for 31 different drug residues (see Table 1). Some of the samples were also tested for additional compounds under FDA European Union Audit Field Assignment. The results of this additional testing are not included in this report as they were not part of the original survey.

B. Data Audit/Review

1. Data Audit

The raw milk samples were tested in three different FDA laboratories. CVM Office of Research Division of Residue Chemistry (DRC) conducted an independent audit of the results to ensure that the ORA laboratories used appropriate methods, that the assignment of all positive findings was based on CVM guidance and that a uniform standard was applied to the interpretation of the data from the three laboratories. For additional information regarding this audit, see http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/ComplianceEnforcement/UCM426355.pdf.

The audit identified some instances where additional data were required to support method validation. Two laboratories completed additional validation work in order to ensure consistency among all of the laboratories. Subsequently, these data were provided by the laboratories and CVM found that the methods were appropriately validated.

As a result of the internal audit, three samples were excluded from the final results and statistical evaluation due to protocol deviations in the laboratory:

- Two samples (SRL 474 and SRL 487) were presumptively positive for Beta-lactams, but the appropriate quantitative method was not used. One sample was identified to have penicillin residues (SRL 487) and the other sample was identified to have both penicillin and ampicillin residues (SRL 474) by Mass Spectrometry LIB method. There are established tolerances for both penicillin and ampicillin in milk. According to the survey assignment, an appropriate quantitative test should have been run after the positive screening results. However, the LIB test was run for a second time and all residues were negative in both samples. Both samples were considered unresolved discrepancies and excluded from statistical analysis.

- One sample (SRL 477) was positive for chloramphenicol when initially evaluated by Mass Spectrometry with the LIB method. Chloramphenicol does not have an established tolerance and is prohibited from use in food-producing animals. According to the survey assignment, for drug residues without an established tolerance or safe level, if it meets the confirmation of identity with the LIB method under CVM Guidance 118, it is to be considered confirmed positive. Although the sample met the criteria for confirmation of identity, a laboratory error resulted in the sample being sent to another laboratory for testing. As a result, the LIB method was repeated with the sample. The second evaluation by Mass Spectrometry found no chloramphenicol present. FDA was not able to resolve this discrepancy; therefore this sample was considered a protocol deviation and was excluded from the final statistical analysis.

In summary, the internal audit confirmed that the ORA laboratories used appropriate methods, that the assignment of all positive findings was based on CVM guidance and that a uniform standard was applied to the interpretation of the data from the three laboratories.

2. Additional Data Review

In addition to the three samples excluded from analysis as a result of the Internal Audit, three additional samples were excluded from the final results and statistical evaluation due to protocol deviations or unresolved discrepancies identified in final quality assurance review:

- Two samples (DEN 575 and DEN 579) were evaluated with the LIB method but were not analyzed for all 31 drugs due to an insufficient amount of raw milk in the sample. All of the drugs that were tested in both samples were negative, but since not all drugs were analyzed it cannot be concluded that these samples were negative. Both samples were considered protocol deviations and excluded from the final results.

- One sample (ARL 242) tested negative for all 31 drugs identified in the survey assignment, including ciproflaxacin, but tested positive for enrofloxacin. Ciprofloxacin is a human drug and also a metabolite of the animal drug enrofloxacin. Although enrofloxacin was not included in the survey assignment, this drug is relevant to the intent of this survey. The discrepancy between the negative ciprofloxacin result and the positive enrofloxacin result could not be resolved; therefore this sample was excluded from the final results.

3. Summary of Data Audit and Additional Review

As a result of the data audit and additional data review, six of the original 1918 samples were excluded from the final results and statistical evaluation due to protocol deviations or unresolved discrepancies. The final determination of three of the 1912 final samples differed from the initial determination as a result of the data audit. See Table B1 for more details.

Table B1: Final Determination of Samples and Residues that Differed from Initial Determinations after Data Audit and Additional Review

Sample Number	Drug	Initial Determination	Notes	Final Determination (by drug)	Final Determination (by sample)
ARL 076	TUL	Positive	Did not meet confirmation of identity in CVM 118	Negative	Negative
ARL 216	FF TIL TUL GEN	Positive Positive Negative Negative	TUL did not meet confirmation of identity in CVM 118. Not enough sample to confirm GEN.	Positive Positive Negative Unknown	Positive
ARL 242	CIP	Negative	UNRESOLVED DISCREPANCY: Negative for ciprofloxacin (a human drug and also a metabolite of the animal drug enrofloxacin) but enrofloxacin was noted to be found at 0.45 ppb. It was not reported as a positive sample by the lab because it is not in the official assignment.	Excluded	Excluded
DEN 510	FF	Positive	Audit noted below level of validation	Negative	Negative
DEN 575		Negative	Milk lost in thawing process	Excluded	Excluded
DEN 579		Negative	Milk lost in thawing process	Excluded	Excluded
DEN 817	FF	Positive	Audit noted below level of validation	Negative	Negative
SRL474	AMP PEN	Positive Positive	UNRESOLVED DISCREPANCY: Survey assignment specifies to quantify drugs with an established tolerance or safe level.	Excluded Excluded	Excluded

Sample Number	Drug	Initial Determination	Notes	Final Determination (by drug)	Final Determination (by sample)
SRL 477	CAP	Positive	PROTOCOL DEVIATION: Survey assignment specifies not to quantify drugs without an established tolerance or safe level.	Excluded	Excluded
SRL 487	PEN	Positive	UNRESOLVED DISCREPANCY: Survey assignment specifies to quantify drugs with an established tolerance or safe level.	Excluded	Excluded