Residues in Milk and Some of Their Implications

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It can be said that the business behind veterinary medicine is run on "notions and emotions." Fads, scares and trends greatly influence food production concepts, animal health regimes and the sale and the distribution of veterinary services and products. Economics drive the dynamic industries of farming and medicine. With the implementation of nutritional science and feed additives, better housing, vaccination and parasite control regimes, and reproductive performance programs, farmers are producing more food per animal and per parcel of land than farmers in the past.

Consumers want food products that are free of residues and contaminants. Due to the use of highly sensitive analytical techniques and equipment today, trace amounts of contaminants and residues can be detected. Our present analytical techniques are more sensitive than our understanding of their "true" impact. The possibility of residue free products today can only be achieved by the use of assured drug free animals. Antibiotics used in the treatment of unhealthy animal, as well as in feed additives, have fallen out of favor with a small sector of the consuming population.

Organic farming has recently become an important concept in the food producing industry. Some consumers prefer known chemically free fruits and vegetables, as well as chemically free products from chemically free animals fed chemically free forages. Foreign trade markets are being closed to U.S. beef producers because of the artificial growth stimulants used in the beef industry. The entire milk industry is faced with the bovine somatotropin (BST) issue. Antibiotics used in the treatment of sick and injured animals leave residues that potentially show up in meat at slaughter and milk before processing. Aflatoxins, possibly from the feeding of improperly stored corn, also can be detected in milk. Residues in food, and in particular milk, can be very detrimental to people.

When residues are reported in milk, great concerns in regard to consumer safety are aroused in the milk industry thus possibly creating an unwanted panic in the market place. This senerio was recently demonstrated in a report published in late 1989 in the *Wall Street Journal.* The FDA's Center for Veterinary Medicine (CVM) spent over $350,000 (approximately $5,000 per sample) running exhaustive analytical tests in an attempt to confirm those results printed in that *Wall Street Journal's* article.[2] Testing included the implicated Charm II® screening test, high-pressure liquid and gas chromatography (with a low to medium ability to identify drugs) and mass spectrophotometry (with a high ability in identifying specific drugs). Seventy milk samples were tested from 14 U.S. cities.

Misleading results were released because a screening test (Charm II®, Penicillin Assays, Inc., Malden, MA) was only used and not followed up by more specific methods. The Charm II® has poor specificity and screens for the presence of beta lactams, aminoglycosides, and sulfonamides. A positive Charm II® test is just an indication that additional testing should be performed. Mass spectrophotometry has the best ability to identify a specific drug. Of those samples that yielded positive results in the FDA's confirmatory tests with the Charm II® test, none of those samples could be confirmed over the "level of concern," which is over 10 ppb for sulfonamides or over 100 ppb tetracycline.

It should also be noted that Stanley Charm, PhD, who markets the Charm II® test himself, does not have approval from the American Organization of Analytical Chemists, and has not submitted the Charm II® test for approval from this group. In 1985, the American Organization of Analytical Chemists did approve the original Charm test.

Louise B. Risk of the Food Animal Concerns Trust stated that veterinarians are only minimally involved in the animal drug residue issue. The problem of drug residues occurs with extra-label use and improper use of drugs by animal owners. This means that producers fail to use drugs properly or fail to observe the recommended withdrawal times. Concerns regarding the results of feeding subtherapeutic concentrations of drugs in the animal's feeds and the consequent public health risk, were also express by Risk.

If anything beneficial can come out of this recent concern for food safety, it would have to be the possible lowering of sulfamethazine (SMZ) tolerance levels made aware by consumer advocates like the Center for Science in the Public Interest. In studies performed by the National Center for Toxicological Research, SMZ (usually given orally as a feed additive) had been shown to be a carcinogen. The

Note: Since this paper was written 10 months ago, some statements may be out of date.

CVM is going to propose that the acceptable tolerance level of SMZ be lowered from its present 100 ppb tolerance level to between 1 and 12 ppb in food animal tissues.

Antibiotic residues in milk not only harm the image of the dairy industry, but are illegal if detected. Methods for the detection of residues are becoming increasingly sensitive, and the reports can be very damaging in this emotional issue. Caution and control must be exercised to protect the reputation of milk and milk products as being wholesome and healthy foods. Exposure of consumers to residues must be prevented by practicing control and client education.

Antibiotics in milk cost the U.S. dairy industry approximately $50 million annually.[4] Current tests can detect penicillin at 0.005 IU/ml. The most commonly associated penicillins are the sustained-release penicillin products. This small concentration of penicillin can cause considerable inhibition of starter activities in cheese and yogurt. Detectable levels of penicillin decrease acid flavor production associated with butter making. Penicillin also reduces the curdling of milk which causes improper ripening of cheeses. Certain antimicrobial agents significantly shift the resistance patterns in the human enteric flora. Some humans are hypersensitive to penicillins, while hypersensitivities and allergic reactions to other antimicrobials are uncommon.

Antibiotics find their way into the total bulk tank milk fluid by individual animal and milking equipment. Antibiotics are administered intramammary, intravenously (injections and infusions), intrauterine, orally or in a feed additive (second most common), and most commonly, intramuscularly. Antibiotics can be detected even if the recommended milk withholding time is followed in some cases.

A sick animal may remove an antibiotic from its body systems (e.g. muscle, mammary gland) at a slower rate than those used as test animals during timed drug studies. Cows treated for mastitis should be milked separately, and have their milk isolated (not included in the bulk tank). Even though these practices are commonly followed, it is still possible to contaminate the milking pipeline and then the bulk tank with a sufficient amount of antibiotics.

With our present methods of detection, thinking that contaminated milk would be diluted out is a fallacy. Calculating it out, a cow with 0.2 IU/ml penicillin would contaminate the milk of a 40 cow herd and still produce a detectable level of 0.005 IU/ml.[4]

As a side note, it has been reported that penicillin and cloxacillin have been detected in the untreated quarters of cows treated for mastitis. Antibiotics administered for dry cow therapy at least 6 weeks before calving did not produce antibiotic residues provided the milk was not shipped for the first four days postpartum. Also, one report showed that dairy calves developed residues when fed milk from treated mastitic cows.[6]

Illegal and improper use of drugs such as benzimidizole anthelmintics can pose a threat to milk consumers. These drugs, when used to control internal parasites, have become an important part of the livestock producing industries. It is also important to educate producers that it is illegal to worm dairy cattle of breeding age because the benzimidazoles and their metabolites may manifest themselves as residues in the milk. Residue monitoring programs have been set up to ensure that animal derived human food does not contain more than 800 ppb in the tissue. Like the Charm tests, screening tests have been developed to test for multiresidue/multidrug class extraction of organophosphates, benzimidazoles, and beta-lactams from meat tissues and milk.

Humans can acquire aflatoxins by the direct ingestion of aflatoxin or by any of its toxic metabolites that are produced by the ubiquitous fungal strains of *Aspergillus flavus* and *A. parasiticus*. These extremely toxic and carcinogenic compounds occur in many grains and foodstufs such as corn, peanuts and rice during the growth, harvest or storage stages. By preventing the contamination of food products or raw materials by means of rapid harvest and drying crops with propely controlled storage, the levels of aflatoxin contamination can be limited, reducing the potential for human exposure.

At present, the permissible level of aflatoxin contamination of agricultural commodities in the U.S. is 20 ppb. The FDA's guideline for fluid milk is appreciably lower, set at 0.5 ppb. Grain products can vary in their final level or concentration of aflatoxin (from less than 1 ppb to 12 ppm) even though mold may be universal to a particular geographic area. The unequal and nonrandom distribution of contamination make quantitation difficult. For example, one peanut in 10,000 may contain aflatoxin at several humdred ppb. Once that single contaminant is ground, blended, and processed (peanut butter, for example) the entire shipment can be considered contaminated.

Aflatoxin B_1 is a carcinogenic mycotoxin commonly found in certain animal feedstuff ingredients. When a cow metabolizes this aflatoxin, the milk may become contaminated with small amounts of aflatoxin M_1.[7] The regulated limits for this aflatoxin range from 10 to 50 ppb, dependent on the milk's end use. Only the lowest limits are allowed for milk intended to be used in infant foods. This is due to the population group's relatively high consumption rate of dairy products, low body weight of this group, and suspected susceptibility of the young to aflatoxin.

Since as little as 2 ppb of aflatoxin in the diet of rats has been found to produce liver tumors following lifetime exposure, the levels of aflatoxin in the diets of humans and especially those with liver cancer should be of serious concern.

Testing of milk samples can be very specific (classical isolation) or broadbased (on-farm or cowside tests) as in those methods that test for multiresidues or multidrugs.

Analytical tests for isolation include the Charm tests and matrix solid-phase dispersion (MSPD), and for determination, liquid chromatography,[5] monoclonal antibody affinity chromatography[3] and mass fraction analysis of whole milk powders[7] certified for aflatoxin content.

The classical testing of milk by monoclonal antibody affinity chromatography direct flurorescence readout confirmed with reverse-phase liquid chromatography yield results are routinely detected at less than 50 parts per trillion of aflatoxin M_1 in 100 ml milk sample.

With multiresidue testing of drug residues in milk, time needed for detection is minimized as a whole range of familial compounds can be identified (e.g. all those beta-lactam rings). With the MSPD method, the chromatogram that is generated gives seven characteristic peaks corresponding to the elution of benzamidizole drugs such as thiabendazole, oxfenbendazole, para-hydroxyfenbendazole, fenbendazole sulfone, mebendazole, albendazole, and fenbendazole.

There are on-farm tests[4] for the presence of acid formation; antibiotic presence (producing an absence of normal bacteria) would fail to turn the indicator strip the desired pH color. There are tests for normal milk enzymes (a positive sample would be inactivated by beta-lactam antibiotics), and tests for bacterial growth. Remember that each test, both specific and broadbased, has its own set of advantages and disadvantages with regard to time, cost, and availability.

Now the purpose of milk is to provide a nutrient rich, easily digestible food source for the newborn. The consumption of the milk (and colostrum) by the newborn offspring is critical to the survival of that offspring. This newborn could be a calf, a seal pup, or even a human baby. Breast feeding has increased in the United States over the last fifteen years. These mothers, along with physicians, are concerned over the possibility of drugs, antibiotics, and environmental and/or occupational chemicals with their persistent residues in the mother's milk, and if found, have the potential to be passed to the offspring.[1]

Pharmaceutical companies spend millions of dollars for drug testing. Physicians discourage the use of drugs by lactating women not because there is data indicating harm, it is rather that there is a lack of information regarding safety. Most safety levels are determined using animal models where an actual or predicted bioconcentration factor can be arrived at. The human factor is achieved by extrapolating a value with a "safety margin factor."

Most environmental chemicals are safely sequestered in the body's adipose stores, even with our efficient hepatic metabolism and urinary excretion. Women that lactate risk the chance of mobilizing these chemicals that the body has stored for their last 15-50 years. Regardless of the time of exposure, lactation provides an avenue where these persistent chemicals can be redistributed and resynthesized in the mammary gland. As a side note, it was reported that people who were poisoned with PCBs and voluntarily fasted for 7-10 days 2-3 years following the exposure showed an elevated blood PCB level.

Milk production puts a stress on the mammary gland. Human milk is 4% fat, rat milk is 15% fat, while reindeer and whale milk is up to 20% fat. If the gland does not receive the triacylglycerides from the diet, the body begins to mobilize its reserves. This is not a real problem in the United States where we have a high fat diet (up to 40%), but in the malnourished countries (low fat diets common) the composition of milk fatty acids resembles that of the adipose tissues stores. The U.S. has strict regulations with the use of environmental and occupational chemicals. Nations that have more relaxed legislation regarding chemical production, application, and disposal may be placing the women who breast feed at a higher risk of potentially harming these women and their babies.

Persistent chemicals of low molecular weight exist most commonly in the nonionized form (hence their lipophilicity), easily pass through the capillary endothelium of the mammary gland, and are more commonly found in the milk than plasma due to their respective fat contents. Highly lipophilic chemicals are mobilized from the fat stores under the conditions which result in lipid depletion, like lactation or fasting. It has been shown that several chemicals are routinely found in human milk: polychlorinated biphenyls (PCBs), total DDT equivalents, hexachlorobenzene, oxychlorodane, and dieldrin.

It has been shown that occupational exposure greatly enhances the chance of elevated milk chemical residues. In one Michigan study performed in 1977-78, over half of the 1,057 human milk samples tested for PCBs (ranged from trace amounts to 5.1 ppm) had milk PCB levels equal to or higher than the FDA tolerance limit set for the sale of cow's milk at that time (1.5 ppm). Tests have been performed worldwide that show DDT and DDE in variable amounts in human milk fat. Although these levels usually meet or exceed the maximum concentration levels that are determined safe for the sale of food, documented toxicities associated with their ingestion are not normally displayed. In fact, it is not possible to determine if the chemical came from the milk or prior exposure, like transplacental transfer. With the number of persistent environmental chemicals, offspring will continue to be exposed to appreciable burdens of chemicals (nursing and environmenal) which may never be eliminated from the body. These lipophilic persistent compounds become concentrated, possibly being passed to the next generation.

Residues in milk, resulting from the environment or possibly introduced by producers and veterinarians, are not desired. Consumers are aware of management practices (antibiotics in the feed, for example) and they equate a negative influence on the use of drugs used in the enhancement of food production. Some of the drugs most commonly detected from dairy cows are penicillins, sulfo-

namides, oxytetracycline, cephapirin, and tylosin.[6] We, as veterinarians, will have to educate producers of the food industry about drug residues. Proper withholding times should be labeled, efforts should be made to identify treated animals, and good medical records should be kept so that the incidence of drug residues is significantly lowered. The owner of the animal bears dominant responsibility for any residues that are created. Even though we cannot change the biological variability of treated animals, veterinarians must do their best to help producers sell safe milk and animals. The association of antimicrobial use and food wholesomeness, accurate or not, will always come back to us as veterinarians. We are in the right position to change this by creating a knowledgeable producer who will produce a consumer friendly product.

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Editor's Note: Mark W. Tengowski was awarded the third prize of $75 for his student clinical paper.