SULFADIMETHOXINE WITHDRAWAL TIME IN LACTATING COWS

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Introduction

Drug residues in milk have received much attention in recent months. Not surprisingly, the residue data collected by different groups, as well as the interpretation of its significance to public health, have been widely divergent. The publicity generated has caused public concerns about the safety and wholesomeness of dairy products.

Because of these concerns and the recognized need of the dairy industry to strictly comply with the FDA requirements regarding allowable tolerance of approved drugs in milk, Hoffmann-La Roche and Rutgers University recently conducted a study to confirm the established milk discard (withdrawal) time following administration of sulfadimethoxine as Albon once-a-day Boluses to lactating dairy cows.

The original sulfadimethoxine tolerance of 10 ppb in milk, established after evaluation of extensive human food safety data by the Agency, remains in effect today. The recent study was conducted to determine if changes in dairy management and assay methods implemented since the original milk discard time was established, had changed the drug withdrawal time required to comply with the established tolerance.

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Experimental Procedures

The biological portion of the study was performed at the Department of Animal Science, Cook College, Rutgers University, under the direction of Dr. G. M. Horton. Twenty-two lactating Holstein cows from the college dairy herd were selected for use in this study. Daily milk production ranged from about 30 to 60 lbs. during the study period. Albon was administered to each cow at the rate of 25 mg/lb body weight on day one, followed by 12.5 mg/lb on days 2 through 5 based on individual body weight. Average body weight was 1,340 lbs.

Milk samples were collected from each cow at each of the two daily milkings during the treatment and milk discard periods when milking was started at approximately 6 a.m. and 3:30 p.m. Duplicate aliquots were collected from the milk meter at each milking and refrigerated until assayed directly or extracted for sulfadimethoxine within 48 hours.

One set of milk samples was assayed for sulfadimethoxine at Hoffmann-La Roche, Nutley, NJ under the direction of Dr. G. Weiss, using the HPLC method, as well as the EZ-Screen* rapid ELISA test. The second set of samples was assayed for sulfadimethoxine at Rutgers University, under the direction of Dr. S. Katz (Department of Biochemistry) using the Charm II test and the Charm Sulfonamide Sensitive Delvo (CIA) test.

The principle of these assay methods is briefly outlined as follows:

High Performance Liquid Chromatography (HPLC): This is the most specific and quantitative of the four assay methods used in this study. It relies upon the affinity between the sulfonamide and a hydrocarbon-like material contained in the separation column through which an aqueous buffer flows. The greater the affinity, the longer the retention time. Detection is by ultraviolet absorption at a fixed wavelength.

This method can be used to separate, identify, and measure different sulfonamides. It is specific for the intact sulfonamide molecule. Its high pressure system allows a high degree of separation of sulfonamides, resulting in very accurate detection. The limit of quantitation for sulfadimethoxine in milk by HPLC is 2 ppb.

Reagents needed for the following three assay methods are available from commercial test kits.

*Environmental Diagnostics Inc.

EZ-Screen Enzyme-Linked Immunosorbent Assay (ELISA): In this assay, the sulfadimethoxine present in the milk sample competes with sulfadimethoxine coupled to an enzyme (peroxidase) for sites on an antibody specific for the heterocyclic moiety of the sulfonamide. This antibody is attached to the test card. Detection is by addition of a substrate which turns blue in the presence of peroxidase.

If 10 ppb or more of sulfadimethoxine are present in the milk, all antibody sites will be occupied, so that no peroxidase can be bound and no color develops. The assay detects sulfadimethoxine and its various metabolites.

The Charm II Receptor Assay: This is a competitive binding assay based on the interaction of antibiotics and sulfonamides present in milk with bacterial receptors specific for classes of antibiotics, or for all sulfonamides as a class. The test measures the amount of radio-labeled antibiotic or sulfonamide bound to the receptor in the presence of the milk sample being tested. The greatest amount of radioactivity is bound to the receptors when no antibiotics or sulfonamides are present in the milk sample. As the amount of antibiotic or sulfonamide in the milk sample increases and binds to the receptor sites, the amount of radiolabeled drug bound to the receptors decreases. This test is not specific for a given antibiotic or sulfonamide. It is intended to detect the presence of classes of these compounds in the milk sample. It detects sulfonamides and their various metabolites. This is the Charm test used by many processors.

The Charm Inhibition Assay (CIA Test): This is a microbial growth inhibition assay in which milk sample impregnated filter discs are placed on the surface of an agar plate inoculated with spores of *Bacillus stearothermophilus.* If antibiotics and/or sulfonamides are present in the sample, colored growth inhibition zones are observed around the discs. The presence of sulfonamides as a class can be confirmed by the absence of inhibition zones when PABA (p-aminobenzoic acid) treated discs are used. This test detects all substances with antibacterial action; classes of antibacterials may be identified through the use of antagonists in the discs. The sensitivity of this method for sulfadimethoxine is 10 ppb.

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Results and Discussion

There were no significant differences between the high- or low-producing cows with regard to sulfadimethoxine (SDM) levels in milk during the treatment and withdrawal periods. The results for both groups have been combined for the purpose of this summary report.

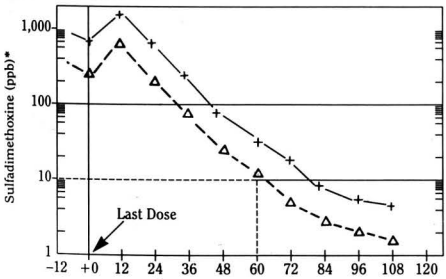
Average sulfadimethoxine concentrations in milk measured by the HPLC method after the last dose are listed in Table 1 and illustrated in Figure 1. When these data were evaluated by the statistical method recommended by the Food and Drug Administration, the previously established milk discard time of 60 hours was confirmed.

Table 1. Average Sulfadimethoxine (SDM) Levels in Milk During Withdrawal Periods.

Average Withdrawal Period After Last Dose (Hrs.)	Milking Period	No. of Cows with >10 ppb SDM*	SDM ppb (std. dev.)
24	2	21	355.95 (115.32)
34	3	21	167.43 (57.76)
48	4	21	54.83 (20.97)
58	5	21	26.81 (11.46)
72	6	13	11.38 (5.47)
82	7	6	8.30 (3.58)
96	8	0	4.60 (2.27)
106	9	0	3.36 (2.21)
120	10	0	2.01 (1.73)
130	11	0	0.78 (1.27)
144	12	0	0.64 (1.06)

*Determined by HPLC assay.

Figure 1. Sulfadimethoxine Levels in Milk During Withdrawal Periods.



+ Average assay value from treated cows.

△ Average assay value using ⅓ dilution factor calculated per FDA guidelines.

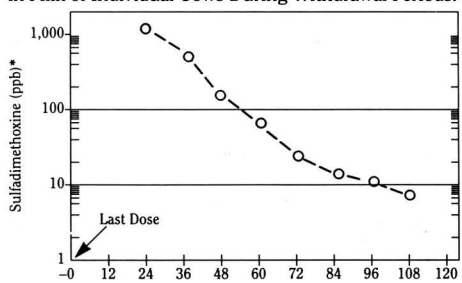
The statistical method used in this analysis assumes that no more than one-third of all cows in a given dairy herd of at least 20 animals are medicated at the same time and provides for a tolerance level of 10 ppb of sulfadimethoxine. If the permitted level of SDM is lowered to 5 ppb, the discard time increases to 84 hours. If the herd conditions assumed by the FDA calculation do not apply, or if milk from individual treated cows is to be marketed without dilution from untreated cows, individual animals must be tested.

Table 2. Calculated Sulfadimethoxine Tolerance Limits in Milk of Individual Cows During Withdrawal Periods.

Withdrawal Period After Last Dose (Hrs.)	Milking Period	SDM (ppb)*
24	2	732.2
34	3	355.9
48	4	123.2
58	5	64.2
72	6	29.2
82	7	20.0
96	8	11.5
106	9	9.2
120	10	5.6
130	11	3.4
144	12	2.9

*Calculated value without dilution factor for 95% confidence that 99% of values from individual cows will fall below this value.

Figure 2. Calculated Sulfadimethoxine Tolerance Limits in Milk of Individual Cows During Withdrawal Periods.



○ Calculated value without dilution factor for 95% confidence that 99% of values from individual cows will fall below this value.

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Tolerance limit SDM residue levels for individual cow milk samples at given withdrawal periods are listed in Table 2 and illustrated in Figure 2. These calculated values represent the upper limits of the individual cow data, providing a margin of safety for a program to help prevent violative SDM residues. The confidence is 95 percent that 99 percent of the values from the individual animals will be lower than these values.

The HPLC assay results correlate well with the calculated values shown in Table 2. The calculated values can be used as guidelines in preventing violative SDM residues in milk from cow herds or individual cows prior to milk pooling.

The results of the EZ-Screen, Charm II, and Charm CIA tests, summarized in Tables 3, 4 and 5 represent the individual cow SDM milk sample at the AM and PM milkings on each day of withdrawal.

Table 3. SDM Clearance from Milk Using EZ-Screen ELISA Test.

Withdrawal Period After Last Dose (Hrs.)	Milking Period	No. of Milk Samples with SDM Residues		
		>10 ppb	Approx. 10 ppb	<10 ppb
24	2	21	0	0
34	3	21	0	0
48	4	21	0	0
58	5	21	0	0
72	6	21	0	0
82	7	13	8	0
96	8	17	4	0
106	9	13	7	1
120	10	5	10	6
130	11	5	7	9
144	12	0	5	16
154	13	0	0	21
168	14	0	1	20

Note that based on individual animal assays, the discard times required for the test values to fall below the 10 ppb of 5 ppb limits is longer for these rapid tests than for the more specific HPLC assay. Nonetheless, this information will be of value to producers involved with bulk milk processors who use these tests. Tables 3, 4 and 5 can provide valuable guidance in determining the appropriate milk discard times when used in conjunction with information on the herd size and number of animals treated.

The results for individual milk samples tested by the EZ-Screen ELISA test are summarized in Table 3. Under the conditions of the test, 13 milkings were needed for all individual samples (no FDA dilution calculation applied) to fall below the 10 ppb limit. Note that this test also measures the antibacterially inactive N-4-acetyl metabolite of sulfadimethoxine.

The results obtained with the Charm II Receptor Assay are summarized in Table 4. This test is sensitive to sulfadimethoxine and to all its metabolites which retain an intact amino group in the 4-position. By this test, milk clearance below 10 ppb required 8 milkings, and below 5 ppb, 13 milkings.

Table 4. SDM Clearance from Milk Using Charm II Receptor Assay.

Withdrawal Period After Last Dose (Hrs.)	Milking Period	No. of Milk Samples with SDM Residues			
		>10 ppb	5-10 ppb	<5 ppb	Negative
24	2	21	0	0	0
34	3	21	0	0	0
48	4	21	0	0	0
58	5	21	0	0	0
72	6	6	15	0	0
82	7	3	11	7	0
96	8	1	7	13	0
106	9	6	11	4	0
120	10	1	5	10	5
130	11	1	4	9	7
144	12	0	1	12	8
154	13	0	1	2	16
168	14	0	0	1	9

Milking No. 13: 19 samples; Milking No. 14: 10 samples.

Table 5. SDM Clearance from Milk Using Charm Inhibition Assay.

Withdrawal Period After Last Dose (Hrs.)	Milking Period	No. of Milk Samples with SDM Residues	
		Positive	Negative
24	2	21	0
34	3	21	0
48	4	21	0
58	5	21	0
72	6	16	5
82	7	10	11
96	8	5	16
106	9	1	20
120	10	3	18
130	11	0	3

Milking No. 11: 3 samples.

The results obtained with the Charm CIA Inhibition Assay are summarized in Table 5. When using this test, clearance of sulfadimethoxine plus all other sensitive inhibitory substances in milk required 10 milkings. Again, this is for individual animals with no FDA dilution calculation applied.

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Summary

Based on the approved tolerance of 10 ppb, the established milk discard time of 60 hours following administration of sulfadimethoxine as Albon once-a-day Boluses to lactating dairy cows at the approved dosage and for the recommended length of time, was confirmed using the High Pressure Liquid Chromatography (HPLC) assay method.

The withdrawal time confirmation is based on the FDA established procedures in which not more than one-third of the milk comes from treated cows and is diluted with not less than two-thirds of the milk, which comes from untreated cows and that milk is processed as pooled bulk product from the whole herd.

Bulk milk processors have the ultimate authority to accept or reject a milk producer's shipment. A processor's decision may be based on the requirement that milk residues may be present at or below the permitted safe tolerance levels set by the Food and Drug Administration, or be totally "residue free" by a test of their choice.

Thus, in addition to complying with the FDA established tolerances, milk producers must also be guided by their knowledge of the processor's requirements regarding residues when discarding milk from treated cows. The veterinarian, when involved, needs to know the proper drug usage, including the rate of drug elimination, and the recommendation to make to the producer when individual or multiple animals in a herd are being treated.

In addition to the HPLC method which was used to reconfirm the Albon withdrawal time, three different commercially available rapid test methods were evaluated for field monitoring of sulfadimethoxine in milk by testing each milk sample in the study. While none of these rapid tests is as specific for sulfadimethoxine as the HPLC method, the rapid test data will enable producers to estimate the milk residue status of each Albon-treated cow. Sulfadimethoxine residues are trace levels in parts per billion concentrations.

Zusammenfassung

Im Hinblick auf die festgelegte Toleranzgrenze von 10 ppb (10^{-7} Volumen-%) wurde die vorgeschriebene Milchverwerfungszeit von 60 Stunden nach einmaliger täglicher Bolusverabreichung von Albon (Sulfadimethoxin) in der zugelassenen Dosierung und für die empfohlene Zeit an Melkkühe mittels Hochleistungs-Flüssigkeits-Chromatographie (HPLC) bestätigt.

Die Festlegung der Verwerfungszeit beruht auf Richtlinien der zentralen Behörde fur Arzneimittelwesen in den USA (FDA). Danach stammt höchstens ein Drittel der Milch von behandelten Kühen, die mit wenigstens zwei Dritteln Milch von unbehandelten Kühen verdünnt sein muss; diese Mischung wird dann als Sammelprodukt der gesamten Herde verarbeitet.

Die Molkerei trifft die endgültige Entscheidung über Annahme oder Ablehnung der Lieferung des Milchproduzenten. Diese Entscheidung kann auf der Grundlage gemacht werden, dass die Restsubstanz in der Milch die von der FDA festgelegte Toleranzgrenze nicht überschreiten oder die Milch vollständig frei von Substanz sein soll. Ausserdem muss der Milchproduzent sich daher beim Verwerfen der Milch behandelter Kühe auch nach den Vorschriften der Molkerei über die Restsubstanz richten. Der evtl. herangezogene Tierarzt muss über die Dosierungs- und Applikationsvorschriften unterrichtet sein, einschliesslich der Eliminationsrate der Substanz und der Empfehlung an den Landwirt, wenn nur ein Tier oder mehrere Tiere der Herde behandelt werden sollen.

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Zusätzlich zur HPLC-Methode zur Bestätigung der Verwerfungszeit von Albon-haltiger Milch wurden in der vorliegenden Untersuchung drei verschiedene auf dem Markt befindliche Schnelltests zwecks Sulfadimethoxin-Feldkontrolle in der Milch untersucht, indem sämtliche Milchproben geprüft wurden. Obwohl keine dieser Schnelltests so spezifisch für Sulfadimethoxin sind wie die HPLC-Methode, eignen sich die Resultate zur Abschätzung der Albon-Restsubstanz in der Milch behandelter Kühe. Sulfadimethoxin-Reste sind Spiegel von Substanzspuren in Konzentrationen von ppb.

Résumé

L'analyse par chromatographie liquide haute performance (CLHP) a confirmé que le lait est en conformité avec la tolérance approuvée de 10^9 parts de résidu si le lait est jeté pendant une période de 60 heures suivant l'administration de sulfadiméthoxine Albon aux vaches laitières à raison d'un bol par jour pour la période recommandée.

L'épreuve employée pour confirmer que la période durant laquelle le lait est jeté assure une concentration de résidu dans les limites prescrites est celle de FDA qui spécifie que pas plus d'un tiers du lait provenant de vaches traitées sera combiné avec au moins deux tiers de lait provenant de vaches nontraitées et que ce mélange sera ajouté à la quantité totale de lait produit par le troupeau et utilisé comme une seule charge.

Les laiteries ont le droit d'accepter ou de refuser une livraison de lait. La décision d'accepter ou de refuser le lait est faite en fonction de la concentration de résidu de sulfadiméthoxine qui doit être au dessous de la limite prescrite par la Food and Drug Administration. Les entreprises laitières peuvent également exiger que le lait est "sans résidu" dans un dosage à leur gré.

Donc le producteur de lait ne doit pas seulement suivre les directives de FDA mais également satisfaire les demandes des laiteries au sujet du volume de lait de vaches traitées à jeter. Le vétérinaire doit être au courant de l'utilisation correcte du médicament et du taux d'élimination du médicament dans l'urine et doit être à même de faire des recommendations pertinentes quand un ou plusieurs animaux d'un troupeau sont en traitement.

Outre la méthode CLHP employée pour confirmer la période durant laquelle le lait doit être jeté, il y a trois tests rapides sur le marché qui ont été évalués quant à leur utilité comme méthode de contrôle sur place du taux de résidus dans le lait. A cet effet tous les échantillons de lait de l'étude en question ont été analysés. Bien qu'aucun de ces tests rapides ne soit aussi spécifique pour la sulfadiméthoxine que la méthode CLHP, ils sont utiles à estimer les résidus dans le lait de chaque vache traitée à l'Albon. Les résidus de sulfadiméthoxine sont de l'ordre de parts pour milliard.

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