ROLE OF VETERINARIANS IN PROVIDING RESIDUE-FREE ANIMAL FOOD

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INTRODUCTION

Veterinarians are not primarily concerned with the increase in production by treating the sick animals and poultry but their important job is to ensure quality (residue free) edible animal products such as milk, meat and eggs to the public. The implementations of WTO regulations demand that veterinarians working in food animal medicine should learn how to avoid drug/chemical residues in food animals and disseminate this information to the farmers to safeguard the health of general public. This issue is also of paramount importance for the veterinarians employed in pharmaceutical and regulatory sectors responsible for assessing the fate of drugs and chemicals that enter the human food chain via the edible products. It is also need of the day that environmentalists, toxicologists and non government organizations (NGO) should pay due attention towards this issue. This is necessary to conduct complete risk assessment, risk management, risk communication studies and implement certain legislative measures to safeguard the public health. This article discusses some important issues in this context such as hazards of drug/chemical residues, modes of chemical exposure, establishment of maximum residue levels (MRL), withdrawal times (WDT) and limitations in residue analysis.

HAZARDS OF DRUG RESIDUES

Potentially, there are two types of hazards relating to drug residues i) direct and short term hazards, and ii) indirect and long term hazards.

Direct and short term hazards

Drugs used in food animals can affect the public health because of their secretion in edible animal tissues in trace amounts usually called residues. For example, oxytetracycline (Salehzadeh *et al*., 2006) and enrofloxacin residues (Salehzadeh *et al*., 2007) have been found above the maximun residual level in chicken tissues. Similarly, diclofenac residues were reported to be the cause of vulture population decline in Pakistan (Oaks *et al*., 2004). Some drugs have the potential to produce toxic reactions in consumers directly; for example, clenbutarol caused illness in 135 peoples as a result of eating contaminated beef in Spain in 1990. Other types of drugs are able to produce allergic or hypersensitivity reactions. For example, 2-β lactam antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms and anaphylaxis at very low doses. Such drugs include the penicillin and cephalosporin groups of antibiotics (Paige *et al*., 1997).

Indirect and long term hazards

Indirect and long term hazards include microbiological effects, carcinogenicity, reproductive effects and teratogenicity. Microbiological effects are one of the major health hazards in human beings. Antibiotic residues consumed along with edible tissues like milk, meat and eggs can produce resistance in bacterial populations in the consumers. This is one of the major reasons of therapeutic failures amongst such peoples. Certain drugs like 3-nitrofurans and nitroimidiazoles can cause cancer in human population. Similarly, some drugs can produce reproductive and teratogenic effects at very low doses consumed for a prolonged period of time. One such example is vaginal clear cell adenocarcinoma and benign structural abnormalities of uterus with diethyl stilbesterol (Sundlof, 1994).

CHEMICAL RESIDUES

Not only the drugs, but chemical residues are also hazardous to the public health. Pesticides are widely used in agriculture. It has been estimated that about three million cases of pesticide poisoning occur worldwide each year, with 220,000 deaths (UNEP, 2004). Majority of these poisonings occur in developing countries due to less protection against exposure, ignorance from health risk and easy access to harmful chemicals. Pesticides have contributed to dramatic increase in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of certain diseases. But pesticides also have harmful effects; they can cause injury to human health as well as to the environment. The range of these adverse health effects includes: acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, dysfunctioning of the immune and endocrine systems, birth defects, and cancer. Problems associated with pesticide hazards to man and the environment are not confined to the developing countries. Developed nations have already suffered these problems, and are still facing some problems in certain locations. For many reasons, the severity of pesticide hazards is much pronounced in third world

countries. A number of long persistent organochlorines and highly toxic organophosphates, which have been banned or severely restricted, are still marketed and used in many developing countries. The misuse of pesticides by concerned individuals, in addition to lack of or weak national controlling plans, is behind the outbreak of adverse effects in developing countries.

A unique mode of chemical exposure

There are basically three ways by which humans/animals are exposed to chemicals. One is ingestion of chemicals that is often very serious and may lead to death. Other is inhalation which is limited to only volatile chemicals. The third route of entry is dermal exposure and this is usually overlooked or underestimated mode of chemical entry to live bodies. But this should be taken as an important way as most of the environmentally toxic chemicals are highly lipophilic (have affinity for lipids). Such chemicals are capable of either binding to skin lipids or can extract the lipids out of the skin. In the first scenario, these chemicals can form skin depots and thus can act as slow releasing formulations. In the second scenario, these chemicals render the skin more permeable to similar or other types of toxic chemicals. This situation results in 2-4 fold increase in the dermal absorption of jet fuel hydrocarbons through the skin that has been previously exposed to jet fuel for 1 and 4 days (Muhammad *et al.,* 2005a, 2005b).

This scenario is important for occupational workers. For example, the crews working in jet engine wear fuel permeable cotton coveralls to reduce the possibility of explosion due to the generation of static electricity associated with more protective clothing. Daily exposure to fuels can result in saturation of the cotton cloth, resulting in an occluded environment for repeated, long-term exposure to the skin during the typical 8 hour workday (Allen *et al*., 2001). Chronic exposure to jet fuel has been shown to cause human liver dysfunction, emotional dysfunction, abnormal electroencephalograms, shortened attention spans, decrease sensorimotor speed and changes in immune functions (Harris *et al*., 2001). Repeated application of petroleum middle distillates to the skin causes chronic irritation and inflammation (Freeman *et al.,* 1990). Fabric soaked with jet fuels for 4 days and evaluated on day 5 produced significant skin damage in pigs (Monteiro-Riviere *et al.*, 2001). The disruption of barrier function of skin, as indicated by an increase in trans-epidermal water loss after exposure to JP-8, might result in increased permeation to its own components and/or other chemicals exposed to skin (Monteiro-Riviere *et al.,* 2001). Pre-exposure of skin to laurocapram, a compound similar to some of the JP-8 performance additives, enhanced the penetration of sodium lauryl sulfate, suggesting that an increase in irritation at the exposed site is possible (Szolar-Platzer *et al.,* 1996). These studies clearly indicate that environmental toxic agents have the potential to cause skin damage and thus render the skin more permeable to other toxic agents.

No doubt, such chemicals are of direct health concern for health regulatory authorities. But at the same time the animals exposed to toxic chemicals may exhibit considerable residues in their edible products (milk, meat, eggs) and are thus an indirect threat to public health.

How these issues can be handled?

Such problems can be resolved by taking into consideration three steps i.e. risk assessment, risk management and risk communication. Basically, risk assessment is a systematic scientific characterization of potential adverse health effects following exposure to hazardous agents. Results from the risk assessment are used to inform risk management, who work with factors like social importance of risk, social acceptability of the risk, economic impacts etc. Finally, risk communication involves making the risk assessment and risk management information comprehensible to lawyers, politicians, judges, environmentalists and community groups. One basic step to build this foundation is the determination of residue levels in our foods.

When the animal is slaughtered or its edible products are collected, there is a legal requirement that drug concentrations in these products are not at levels greater than those established as safe by the relevant regulatory authority in the country of origin. In many countries of the world, this upper level is referred to as the maximum residue level (MRL), while in United States it is termed as tolerance (Riviere, 1999).

MRLs and tolerances are established by regulatory authorities based on many factors primarily relating to the safety of the animal product to the consumer, the usage pattern of the compound (pesticide in the field), and analytical methodology. The major determining factor is food safety. In this context, the focus is the length of time after discontinuation of drug administration or chemical exposure required to allow a tissue to deplete to a concentration below the MRL (Fitzpatrick *et al*., 1995). This is the pre-slaughter meat withdrawal time (WDT; Tables 1 and 2). If the matrix is milk, then the parameter of interest is the milk discard interval (MDI). This will be interesting to know that how is the tissue tolerance or MRL established.

Maximum residue level

The MRL or tolerance is the target concentration in a residue-depletion study. It should be established purely on the basis of safety to the person consuming the product and has no pharmacodynamic reality in the animal to which the drug has been administered. Tissue tolerances are normally established in fat, milk, muscle, liver, kidney, skin, or sometime meat by-products.

Table 1: Withdrawal times of some important drugs for dairy cows

Drugs	*Preslaughter withdrawal (days)	*Milk discard (days)
Injectables		
Ampicillin trihydrate	6	2
Amoxicillin trihydrate	25	4
Ceftiofur hydrochloride	2	0
Ceftiofur sodium	0	0
Erythromycin	14	3
Furosemide	2	2
Hydrochlorothiazide	0	3
Isoflupredone acetate	7	0
Oxytetracycline	28	4
Procaine penicillin G	10	2
Sulfadimethoxine	5	2.5
Tripelennamine	4	1
Oral medications		
Chlorothiazide (bolus)	0	3
Fenbendazole (suspension)	8	0
(paste)	8	0
Fenbendazole (blocks)	13	0
Morantel tartrate (feed)	14	0
Trichlormethazide + dexamethasone	0	3
Topical treatments (Dry cows only)		
Famphur	35	Do not use within 21 days of application.
Fenthion	35	Do not use within 28 days of application.
Phosmet	3	Do not use within 28 days of application.
Coumaphos	0	Do not use within 14 days of application.
Intramammary application (Dry cow therapy)		
Benzathine cephapirin	42	72 hours
Benzathine cloxacillin	30	-
Erythromycin	14	36 hours
Dihydrostreptomycin sulfate + procaine penicillin G	60	Within 96 h after calving
Novobiocin	30	-
Novobiocin sodium + procaine penicillin	30	Within 72 h after calving
Procaine penicillin G	4	Within 72 h after calving
Intramammary Application (Lactating cow therapy)		
Amoxicillin	12	60 hours
Erythromycin	14	36 hours
Novobiocin + procaine penicillin G	15	72 hours
Pirlimycin	28	36 hours
Potassium hetacillin	10	72 hours
Procaine penicillin G	3	60 hours
Salicylic acid	-	48 hours
Sodium cephapirin	4	96 hours
Sodium cloxacillin	10	48 hours

* Data obtained from Mississippi State University Extension Services, USA.

Table 2: Withdrawal times of some important drugs for sheep and goats

Drugs	*Preslaughter withdrawal (days)
Oral medications	
Albendazole	7
Ivermectin	11
Levamisole hydrochloride	3
Neomycin sulfate	2
Feed medications	
Chlortetracycline	0
Decoquinate	0
Lasalocid	0
Neomycin sulfate	2
Water medications	
Neomycin sulfate	2
Oxytetracycline	5
Injectables	
Sodium selenite (Vitamine E/selenium)	14
Ceftiofur sodium	0
Erythromycin	3
Procaine penicillin	9
Topical treatments	
Cyano (3-phenoxyphenyl) methyl- ectrin 4-chloro-alpha-(1-methylethyl) benzeneacetate	2
Permethrin	0
Miscellaneous	
Zeranol (implant pellet)	40
Drugs labeled for use in goats	
Decoquinate (feed)	0
Monensin (feed)	0
Neomycin sulfate (feed)	3
Neomycin sulfate (water)	3
Neomycin sulfate (oral)	3

* Data obtained from Mississippi State University Extension Services, USA.

The first step in calculating the tolerance is to determine the safe concentration of drug that could be consumed by individuals eating the animal products:

$$\text{Safe concentration} = (\text{ADI})\,(\text{Body weight})/\text{Food consumption factor}$$

In this equation, ADI refers to acceptable daily intake which is the maximum amount of chemical (mg/kg) that may be consumed daily over a lifetime without producing an adverse effect. Body weight is the average weight of humans consuming the product (usually assumed to be 60 Kg). The food consumption factor is the amount of edible product estimated to be consumed daily by an individual. The food consumption factor is based upon the average individual's daily intake of different types of foods. The Food and Drug Administration (FDA) and other regulatory agencies have tabulated food-specific consumption factors. Examples (Kg consumed per day) are 0.3 for muscle, 0.1 for liver, 0.05 for kidney, 0.05 for fat, and 1.5 for milk in USA (Riviere, 1999). The milk consumption in children is especially high since the total diet for an infant may entirely be the milk. Other countries use similar food consumption factors but distribute the ADI based on independent organ consumption data.

The most controversial component in this calculation is the establishment of ADI, which involves the risk assessment extrapolation from laboratory animal toxicology studies. ADI is estimated as a fraction of the no-observed-adverse-effect-level (NOAEL) determined from standardized long-term laboratory animal toxicological studies conducted in at least two animal species. The NOAEL is then divided by a safety factor ranging from 100 to 1000, depending on the nature of the compound's toxicology or the strength of the data. Part of this factor is to account for the vagaries of interspecies extrapolations (rodent to human) and to be conservative in the face of more acute and serious toxicity (teratogens, hypersensitivity, etc.). The FDA uses a safety factor of 100 for a chronic study and 1000 for a 90-day toxicity study. This can be appreciated that the safety factor is greater when there is evidence of teratogenic effects or when a more economical subchronic (90-day) study is submitted in place of a more complete chronic study. This latter factor alone can result in a 10 fold lower tolerance (and hence longer withdrawal time) being established for a product supported by 90-day studies compared to the identical formulation supported by a chronic study (Concordet and Toutain, 1997). Thus, a subjective bias is directly built into the analysis that is independent of the actual toxicological properties of the compound.

The final step is to establish the tolerance. The safe concentration is based upon the total concentration of drug (total residues), which includes the parent drug and any metabolites. Some special regulations apply for covalently bound residues (Lu *et al*., 1988). Depending on the drug, bound residues may be included as either a component of the total or a fraction removed from consideration. A marker residue is now selected that has a defined relationship to the total residues. If the drug is not metabolized, the safe concentration becomes the tolerance. If the drug is metabolized, the tolerance will be a fraction of the safe concentration. In many other countries, the process of establishing MRLs is very similar to the above mentioned protocols with very few exceptions.

Psychological/sociological considerations

In order to establish a firm base to resolve the above mentioned issues, certain aesthetic considera-tions, risks perceived by the public, sensitive populations and issues, international relations and trade barriers have to be considered. There is an urgent need

for comprehensive anthropological studies to prioritize the issues and their solutions.

Limitations in residue analysis

One basic limitation to conduct residue and risk analysis is the detection of chemical residues in edible animal products. With out accurate detection, exact risk is impossible to assess. This process needs highly qualified expertise, sensitive instruments and modern analytical techniques. High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) are sensitive instruments while Solid Phase Micro-extraction (SPME) and Microdialysis are modern analytical techniques used for residue analysis.

Conclusions

Veterinarians must be well aware of the importance of drug/chemical residues in the food animals and their possible risk to the general public. They must have updated information about the proper withdrawal times of all the drugs/chemicals used in their areas of practice. They must extend this information to the livestock and poultry farmers for the production of residue free edible animal products like milk, meet and eggs. For residue analysis, trained manpower are needed. In this regard, the availability of sensitive equipment and modern analytical techniques are of paramount importance.

REFERENCES

Allen, D. G., J. E. Riviere and N. A. Monteiro-Riviere, 2001. Cytokine induction as a measure of cutaneous toxicity in primary and immortalized porcine keratinocytes exposed to jet fuels, and their relationship to normal human epidermal keratinocytes. Toxicol. Lett., 119: 209-217.

Concordet, D. and P. L. Toutain, 1997. The withdrawal time estimation of veterinary drugs: A non-parametric approach. J. Vet. Pharmacol. Therap., 20: 374-379.

Fitzpatrick, S. C., S. D. Brynes and G. B. Guest, 1995. Dietary intake estimates as a means to the harmonization of maximum residue levels for veterinary drugs. J. Vet. Pharmacol. Therap., 18: 325-327.

Freeman, J. J., R. H. McKee, R. D. Phillips, R. T. Plutnick, R. A. Scala and L. J. Ackerman, 1990. A 90-day toxicity study of the effects of petroleum middle distillates on the skin of C_3H mice. Toxicol. Ind. Health, 6(3-4): 475-491.

Harris, D. T., D. Sakiestewa, D. Titone, R. F. Robledo, R. S. Young and M. Witten, 2001. Jet fuel induced immunotoxicity. Toxicol. Ind. Health, 16(7-8): 261-265.

Lu, A. Y. H., G. T. Miwa and P. G. Wislocki, 1988. Toxicological significance of covalently bound residues. Rev. Biochem. Toxicol., 9: 1-27.

Monteiro-Riviere, N. A., A. O. Inman and J. E. Riviere, 2001. The effects of short term high dose and low dose dermal exposure to Jet A, JP-8, and JP-8 + 100 jet fuels. J. Appl. Toxicol., 21: 485-494.

Muhammad, F., N. A. Monteiro-Riviere and J. E. Riviere, 2005a. Comparative *in vivo* toxicity of topical JP-8 jet fuel and its individual hydrocarbon components: Identification of tridecane and tetradecane as key constituents responsible for dermal irritation. Toxicologic Pathol., 33: 258-266.

Muhammad, F., N. A. Monteiro-Riviere, R. E. Baynes and J. E. Riviere, 2005b. *In vivo* effect of jet fuel exposure on subsequent in vitro dermal absorption of individual aromatic and aliphatic hydrocarbons constituents. J. Toxicol. Environ. Health, 68: 719-737.

Oaks, J. L., M. Gilbert, M. Z. Virani, R. T. Watson, C. U. Meteyar, B. A. Rideout, H. L. Shivaprasad, S. Ahmed, M. Jamshed, I. Chaudhry, M. Arshad, S. Mahmood, A. Ali and A. A. Khan, 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature, 427: 630-633.

Paige, J. C., L. Tollefson and M. Miller, 1997. Public health impact on drug residues in animal tissues. Vet. Human Toxicol., 9: 1-27.

Riviere, J. E., 1999. Comparative Pharmacokinetics, Principles, Techniques and Applications. Iowa State Univ. Press, Ames, Iowa, USA.

Salehzadeh, F., R. Madani, A. Salehzadeh, N. Rokni and F. Golchinefar, 2006. Oxytetracycline residue in chicken tissues from Tehran slaughterhouses in Iran. Pakistan J. Nutr., 5(4): 377-381.

Salehzadeh, F., A. Salehzadeh, N. Rokni, R. Madani and F. Golchinefar, 2007. Enrofloxacin residue in chicken tissues from Tehran slaughterhouses in Iran. Pakistan J. Nutr., 6(4): 409-413.

Sundlof, S. F., 1994. Human risks associated with drug residues in animal derived food. J. Agrimedicine, 1: 5-22.

Szolar-Platzer, C., S. Patil and H. I. Maibach, 1996. Effect of topical laurocapram (Azone) on the *in vitro* percutaneous permeation of sodium lauryl sulfate using human skin. Acta Derm. Venereol., 76: 182-185.

UNEP, 2004. The Chemical Programme of the United Nations Environment Programme (UNEP chemicals). International Environment House, 11-13 Chen des Anemones, CH-1219 Chatelaine, Switzwerland.