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VI/3 Carcinogenicity Studies in Animals Relevant to the Use of Anabolic Agents in Animal Production

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Summary

It has long been known that certain estrogens and testosterone may increase, or sometimes decrease, the incidence of neoplasmas in laboratory animals. They probably act by switching-on inappropriate genetic information or switching-off appropriate genetic information contained in nucleic acids. For instance, they may switch-on in adult animals information which is relevant only to a certain stage of embryogenesis or they may facilitate the expression of oncogenic viruses which would otherwise lay harmlessly dormant. The situation is rendered complex because an effect on one endocrine gland leads to effects on others so that factors which favour tumour development may result indirectly from administration of an anabolic or other hormonal agent. Two kinds of neoplasm are now known to be associated with human exposure to anabolic agents: vaginal adenocarcinoma and liver-cell tumours. Tumours of both kinds are among the spectrum of neoplasms that has been seen in laboratory animals exposed to agents of the same kind. In both animals and man there is evidence that tumours arising in response to anabolic agents are sometimes, initially at least, hormone-dependent.

The evidence that 17β-estradiol, diethylstilbestrol, chlormadinone, and testosterone are carcinogenic for laboratory animals is briefly reviewed and the reader's attention is directed towards the 1974 IARC Monograph on the evaluation of sex hormones for carcinogenic risk to man where the same evidence is reviewed more extensively.

The significance for man of the results of studies on laboratory animals is discussed with special reference to the use of anabolic agents in meat production. Non-residue uses are to be preferred, as are naturally-occurring agents as opposed to compounds which do not occur in nature. More information is needed concerning the possible effects of prolonged exposure to *very low doses* of anabolic agents.

1. Introduction

There can be few more complex problems in carcinogenesis than those which concern the effects on cancer incidence of sex hormones, both natural and unnatural. It was undoubtedly foolish of me if not presumptive to have accepted to talk on this subject at only 3 weeks notice, because the acknowledged expert on the subject, Dr. J.W. Jull, had suddenly and sadly died. My direct experience of carcinogenesis studies in this field is extremely limited. Indeed it might be said that my only qualification to speak on the subject at all is a personal one that I share with about half the world's population, namely as enthusiastic producer of testosterone and conjugated estrogens!

In setting about my task there seemed to me to be six important questions. These are set out in Table 1.

Table 1 Six Important Questions

1. Are anabolic agents carcinogens?
2. Are they carcinogenic for man?
3. Is carcinogenicity related to hormone activity?
4. If high doses cause cancer, should low doses be regarded as carcinogenic?
5. Should unnatural agents be regarded with more suspicion than natural ones?
6. How may the safety-in-use of an anabolic agent be ascertained?

2. Definitions and Mechanisms

Before discussing the available evidence, it is necessary for me to discuss briefly the nature of carcinogenesis. For the purposes of the present paper, I use the term "carcinogen" to describe *"An agent which under defined conditions increases the age-standardized risk of development of, or death from, one or other form of malignant disease as compared with matched control subjects (or animals in the case of animal experiments) not exposed to the agent."* This definition takes into account the important fact that most, if not all, forms of malignant disease occur, apparently spontaneously, at some level of incidence. The wording, however, leaves entirely open the question of the mechanisms involved. It is currently fashionable to regard the many mechanisms involved in *carcinogenesis* as falling into two groups: those involving changes in the genetic apparatus of cells, e.g. alteration in or additions to the nucleic acid complement, and a wide variety of mechanisms which do not involve any genetic-type change see Figure 1. Most authorities accept that where hormones increase cancer risk they do so by the latter kind of mechanism. Hormones can only switch on information which is already present in cells. They do not change information or add to it. The effects that a hormone may have in an animal are limited by the information available to be switched on in the cells of that animal. All body cells carry much more genetic information than they express and some of the normally suppressed information is potentially dangerous to the whole organism if it is switched on. If a hormone is introduced which leads to the switching-on of this information, then conditions such as cancer, which threaten the life of the whole organism may arise. The potentially dangerous information in this context may be plans for organs the development of which is normally completed early in embryonic life, or the blueprints for organs that are only appropriate to members of the opposite sex. But the potentially dangerous information might also be that contained in the nucleic acids of oncogenic viruses or once-normal cellular DNA that has been damaged by previous exposure to an environmental toxin. An

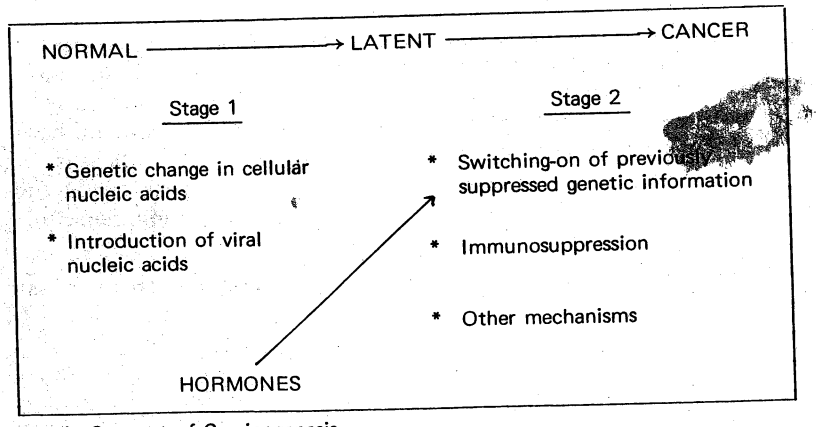


Fig. 1 Concept of Carcinogenesis

overview of the vast literature on the carcinogenic effects of various hormones encourages the view that the switching-on of potentially dangerous information is commonly involved in hormonal carcinogenesis. In laboratory animals some of the most readily produced neoplasms e.g. the mammary tumours and lymphomas of mice are associated with the presence of oncogenic viruses. However, nothing about this complex subject is simple. The different endocrine glands of the body interact such that the secretion(s) of one suppress or enhance those of others and normal status is maintained by numerous complementary negative-feedback mechanisms. The removal of a gland or the introduction of an exogenous hormone, therefore, may have repercussions throughout the endocrine system and effects may be seen that are not directly attributable to the gland removed or the hormone administered.

It is a feature of some of the neoplasms that arise in association with hormone treatment in laboratory animals that they only continue to thrive as long as hormone treatment continues. Withdrawal of the hormone leads to the regression and eventual disappearance of even apparently malignant (i.e. invasive) tumours of this kind – which are referred to as "hormone-dependent". Sometimes, however, tumours which are initially hormone-dependent eventually, as a result of tumour progression, become hormone-independent.

These features of hormone carcinogenesis are summarized in Table 2. I have refrained from attempting to support the above rather general statements by extensive quotations from the published literature because the subject has been recently and excellently reviewed in Volume 6 of the monographs prepared by the International Agency for Research on Cancer[1].

Table 2 Some features of Hormone Carcinogenesis

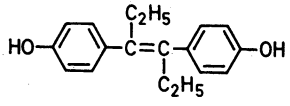
1. Hormone-promoted cancers occur 'spontaneously'.
2. Hormones – exogenous or endogenous – may cause cancer or predispose to it by:
   (i) Switching-on information relevant to embryogenesis.
   (ii) Switching-on information relevant to the opposite sex.
   (iii) Switching-on information in oncogenic viruses.
   (iv) Interference with normal negative feedback mechanism leading to unremitting growth stimulation of particular tissues.
   (v) Indirectly via effects on one of more endocrine glands.
3. Hormones may **reduce** cancer risk.
4. Hormone-promoted tumours may be hormone-dependent.

3. Carcinogenesis in Laboratory Animals by Certain Anabolic Steroids and by Diethylstilbestrol

In this section I propose to review briefly evidence from laboratory studies on certain anabolic steroids which have been used in meat production namely:

Diethylstilbestrol
17β-Estradiol
Chlormadinone
Testosterone

3.1. Diethylstilbestrol



This non-steroidal estrogenic agent which does not occur naturally has been widely used as a feed additive or as an implant into the ear to promote growth and increase

feed efficiency in beef cattle and sheep. It has also been widely used as an implant, usually in the neck, to caponise male chickens and to promote their growth. Three years ago these uses were banned in the United States, but the ban was successfully challenged in the Courts. It is thus still used in the United States and in some other countries. One of the reasons for the imposition of the ban was that residues of stilbestrol could be detected in the meat of treated animals and birds.

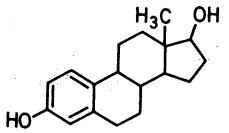
The oestrogenic properties of stilbestrol were first described by Dodds et al.[2] and it was only 3 years later than this that the first reports that the agent may increase cancer incidence in animals in certain circumstances began to appear. Shimkin and Grady[3] observed an increased incidence of virus-associated mammary tumours in mice given stilbestrol repeatedly by stomach tube. According to Ball et al.[4], however this effect can (in virgin female Strain A mice, at least) be prevented by severe dietary restriction. According to Gass et al.[5] a dietary level as low as 50 ppb of stilbestrol fed continuously was sufficient to increase significantly the incidence of mammary tumours in C3H female mice, but intermittent feeding of stilbestrol proved much less effective than continuous feeding[6]. Murphy and Sturm[7] reported that stilbestrol increased the incidence of malignant lymphoma in male mice of a high spontaneous lymphoma strain, and Andervont et al.[8] recorded a high incidence of interstitial cell tumours of the testis in BALB/c mice which bore subcutaneous implants of stilbestrol. An effect in the opposite direction was a reduction in liver cell adenomas – a commonly occurring neoplasm – in male mice of some strains in response to stilbestrol[9].

Perhaps of greater relevance to man were the reports by Dunn and Green[10] and Gardner[11] of an increased incidence of cancers of the cervix and vagina in BALB/c mice in response to stilbestrol. In the case of these tumours there is no evidence that an oncogenic virus is involved.

Stilbestrol, like estradiol, gives rise to kidney tumours in male hamsters[12], and increases the incidence of mammary tumours in some strains of rat[13].

The subcutaneous implantation of pellets of stilbestrol in squirrel monkeys gives rise to proliferative changes and mesotheliomatous neoplasms of the uterine serosa[14].

3.2. 17β-Estradiol



This naturally-occurring estrogen is widely used, in the form of implants, to promote growth and increase feed efficiency in heifers, lambs and steers. It is also given to prospective roasting chickens to aid uniformity in fat distribution. If properly used, no residues of the hormone are found in the meat of the treated animals and birds.

17β-Estradiol has been shown to increase the incidence of 2 kinds of oncogenic virus-associated neoplasms in mice – mammary tumours and malignant lymphoma[15-17]. Kirschbaum et al.[18] reported that X-rays and estradiol acted synergistically to produce mammary tumours in one strain of mice.

Tumours which might be secondary to interference with the general hormonal milieu have been seen in the form of pituitary tumours and interstitial tumours of the testis in various strains of mice[16,19].

An effect in the opposite direction is seen in the case of liver-cell adenomas. The incidence of this common neoplasm in male mice is reduced by estrogens generally[20].

Invasive cancers of the cervix and vagina were reported in mice given once-weekly injections of 16.6 or 25 μg estradiol benzoate by Pan and Gardner[21] and other workers have reported vaginal hyperplasia, persistant cornification and lesions resembling early invasive epidermoid carcinomas in mice given relatively high doses of estradiol by subcutaneous injection during the first few days of life[22,23].

Estradiol may increase the incidence of pituitary and mammary tumours in rats[24,25] and in guinea-pigs a variety of hormone-dependent tumours of the uterus and other abdominal organs has been seen in response to the agent[26,27]. The main target organ for carcinogenesis by estrogens, including estradiol, in the male hamster and ovariectomised female hamster is the kidney whereas its administration to intact females is without effect. The kidney tumours which arise are primitive in appearance and initially hormone-dependent[28].

It is not difficult to see from a brief summary of the evidence for carcinogenicity in respect of the unnatural agent, diethylstilbestrol, and of the natural agent 17β-estradiol (Tables 3 and 4) that there is a close similarity between them in the kinds of tumour they predispose to in animals.

Table 3 Evidence that Diethylstilbestrol is Carcinogenic for Laboratory Animals

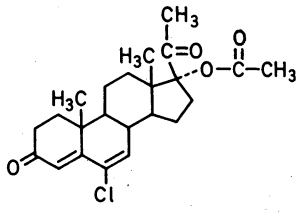
Species	Neoplasm	Oncogenic virus Implicated	Hormone-dependency
Mouse	Mammary	+	
	Lymphoma	+	
	Testis		
	Cervix and Vagina		
Rat	Mammary		
Male Hamster Castrated female Hamster	Kidney		+
Squirrel Monkey	Mesothelioma		+

N.B. Reduction in liver-cell tumours in one strain of mice.

Table 4 Evidence that 17β-estradiol is carcinogenic for laboratory animals

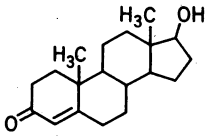
Species	Neoplasm	Oncogenic virus Implicated	Hormone-dependency
Mouse	Mammary	+	
	Lymphoma	+	
	Pituitary		
	Testis		
	Liver-cell		
	Cervix and Vagina		
Rat	Pituitary		
	Mammary		
Guinea-pig	Uterus		+
Male Hamster Castrated female Hamster	Kidney		+

3.3. Chlormadinone Acetate



This progestational agent which does not occur in nature has been allowed in cattle feed under certain restrictions for the purpose of synchronising estrus in beef heifers and beef cows. It has not been used because of any anabolic activity it may have. Given alone, chlormadinone acetate did not enhance the incidence of any form of neoplasm in rats or mice even at doses at high as 200–400 times those given to women for contraceptive purposes[29]. Mammary nodules, some of which were considered to be neoplasms were, however, seen in female dogs fed the agent for up to 2 years[30,31].

3.4. Testosterone



This naturally-occurring androgen is, or has been, widely used in combination with estrogens, such as estradiol of stilbestrol, to promote growth and increase feed efficiency in heifers.

There is much evidence that androgens in general, and testosterone in particular, favour the development of liver-cell tumours in mice. In virtually all mouse strains the spontaneous incidence of such neoplasms is higher in males than in females and their incidence in females is enhanced by androgen administration[20]. By contrast testosterone was found to reduce the incidence of mammary tumours in mature virgin female mice[32,33] and of malignant lymphoma in ovariectomized females[34].

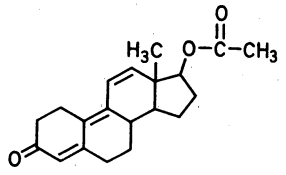
The picture when testosterone is given to mice very early in life tends to be rather different. Testosterone given to new-born female BALB/c mice gave rise to a high incidence both of vaginal tumours[23] and of mammary tumours[35]. Thus, in these immature animals, testosterone had much the same effect as 17β-estradiol.

A parallel with the effects of estradiol was also seen, however, in female mice that received testosterone in high dosage throughout life in the form of a high incidence of cervical tumours many of which were invasive and showed metastasis to the lungs[36].

Since androgens are used together with estrogens for growth-promoting purposes it is relevant to look at the results of studies in which animals have been exposed to combinations of the two agents. Kirkman[37], Riviere et al.[38], and Kirkman and Algard[39] saw endometrial tumours, tumours of the vas deferens and seminal vesicles and basal-cell epitheliomas in hamsters so treated.

3.5. Other Agents

Many other anabolic steroids are available for verterinary use. According to Heitzman[40] most of them are marketed as therapeutic agents for disease conditions and not on account of their growth promoting properties relevant to meat and milk production. One such agent is *trenbolone acetate.*



Heitzman says of this agent that its use gives rise to only low residues in meat and milk, that it is almost inactive orally and is destroyed by heating. "Therefore", he deduces "androgenic side effects in human should be negligible". Such a deduction, however, should not be regarded as an adequate substitute for a full appraisal of its potential toxicity and for this appraisal there would have to be data from appropriate long-term studies on laboratory animals.

Zeranol, a non-steroidal estrogen, has been widely used for improving growth and feed efficiency in lambs and beef cattle. According to Umberger[41], animal studies undertaken so far have shown no indication of carcinogenicity. It is difficult to believe, however, that evidence of carcinogenicity will not be obtained if enough tests are carried out at sufficiently high doses.

Melengestrol acetate (MGA), a progestational agent, according to Gerrits[42], exhibits growth promoting activity when fed to intact animals by allowing continuous endogenous estrogen production. The compound has no effect in ovariectomised females. In this case an appropriate test for carcinogenicity would be one in which the administration of MGA led to continuous endogenous estrogen excretion in the test animals.

4. Evidence of carcinogenicity in Man

It is now clear that the human animal is not immune to carcinogenesis by anabolic agents.

Several groups[43-45] reported between them 13 cases of adenocarcinoma of the vagina in adolescent girls – an exceptionally rare tumour. In all 13 cases there was a history that their mothers had been given diethylstilbestrol during pregnancy. The treatment was given to prevent abortion. The doses of stilbestrol given to the mothers varied but were in all cases massive. They were based on a regimen proposed by Smith[46] which started with an oral dose of 5 mg per day during the 6th week of pregnancy and increased to as much as 150 mg per day by the 35th week of pregnancy. The highest of these doses amounted to approximately 2 mg/kg/day. In the United States during the 1940's and 1950's of the order of 200.000 pregnant women were treated in this way. At the Boston Lying-In Hospital, for instance, some 841 women were so treated between 1947 and 1958 at a Diethylstilbestrol Clinic[47]. At present it would seem that only about 1 in 1.000 of the daughters of treated women develop vaginal carcinomas by the age of 18–20[48], however, abnormalities of the cervix/or vaginal epithelium (e.g. adenosis) have been found in more than 50% of the daughters – and these changes may predispose to cancer as the girls get older. Thus the full extent of the cancer risk cannot at the present time be ascertained. So far there have been no reports of increased cancer risk in the sons of treated women, but again, this does not mean that cancer incidence in later life will not be affected in them. It is interesting that the risk of vaginal cancer was higher in the daughters of women who started on diethylstilbestrol during the first trimester than those of mothers who did not begin to receive the drug until later in pregnancy.

This vogue for treating pregnant women with stilbestrol happened, of course, before the thalidomide disaster, before teratogenicity testing was added to the requirements for drugs, food additives, pesticides, etc. before Watson and Crick described the structure of DNA, before the birth of molecular biology and before anyone had invented the term "Transplacental Carcinogenesis". Today, to use a potent drug, such as stilbestrol, in the way it was given to pregnant women during the 1940's and 1950's would be regarded as irresponsible in the extreme!

Several reports indicating an association between exposure to anabolic and contraceptive steroid hormones and liver-cell adenomas in humans constitute the second happening which

has brought the subject into prominence. Bernstein et al.[49] reported vascular liver changes (peliosis) and a well-differentiated liver cell tumour in a patient treated with oxymetholone and 2 years later Baum et al.[50] described a series of 6 cases of liver-cell adenoma in patients receiving oral contraceptives. A further 10 or so similar cases had been reported by other workers by the middle of 1974[51-57]. In addition Thalassinos et al.[58] described a case of primary liver cancer in a woman of 30 who had been treated before and during pregnancy with estrogens and Aldercreutz and Tenhunen[59], Johnson et al.[60] and Farrell et al.[61] have between them recorded 8 cases of primary liver cancer in persons treated with androgenic anabolic steroids.

Two kinds of tumour-production in laboratory animals would seem at first sight to be relevant to humans, namely, vaginal and liver-cell neoplasia. Thus, the development of vaginal neoplasms in mice in response to estrogens and testosterone, particularly if animals are exposed when they are newly born, looks to be a fairly exact model for man. It is rather less clear whether the activity of these same hormones in increasing the incidence of liver-cell tumours, particularly in mice, is a model for the production of liver-cell tumours in humans by certain androgenic or progestational agents. The fact that tumours of this kind may occur spontaneously in very high incidence in some mouse strains, especially in males has no obvious parallel in man nor does the fact that numerous factors, such as microbial status and quantity and quality of diet, may profoundly influence liver tumour incidence[62,63].

Hormone-dependancy would seem to be a feature of the liver tumours associated with exposure to anabolic steroids in humans. Farrell et al.[61] reported regression in 2 out or 3 males with hepato-cellular carcinoma when treatment with anabolic steroids was stopped. Regression was also observed in one similar case by Johnson et al.[60]. It should also be noted, however, that in one of the cases of Farrell et al.[61] regression did not occur on withdrawal of treatment. The man in question had been taking 50 mg methyltestosterone tablets daily for 8 years and receiving injections of testosterone propionate (50 mg once monthly) because of cryptorchidisin. Whether his liver tumour was hormone-dependent when it first arose and only later became hormone-independent is not known.

5. Discussion

It would now be appropriate to look again at the six questions listed in Table 1. Obviously the answer to the Question 1 is "Yes". In the case of diethylstilbestrol the answer to Question 2 is also "Yes". Moreover, in the light of the similarity of the animal data for the two substances, summarized in Tables 3 and 4, I have no doubt that 17β-estradiol would give rise to vaginal changes and neoplasms in the daughters of women given it in high doses during pregnancy. However, the affirmative answers to questions 1 and 2 help us in no way to decide whether anabolic agents should be used in meat production. In this connection we must first throw aside the well meaning but clearly inappropriate constraints of the Delaney Amendment. There is a good theoretical case for trying to apply the Delaney concept to Stage 1 carcinogens (see Figure 1) but it clearly is not appropriate to think in terms of zero tolerance for agents which humans and other animals need for survival and secrete endogenously. Earlier in this Symposium reference has been made to the modified Mantel-Bryan procedure. With respect to those eminent mathematicians the problem which concerns us – namely the question of whether it is safe to use anabolic agents in meat production – is a biological one and not a mathematical one. There is no evidence that any of the agents that concerns us has carcinogenic activity which is not directly related to its hormonal activity and my reading of the available literature has brought to light no example of an anabolic agent increasing tumour incidence when given in doses inadequate to produce obvious hormonal effects. The only possible exception is

a report by Gass et al.[5] of an increased incidence of mammary tumours in C3H female mice given as little as 50 ppb diethylstilbestrol in the diet. This finding needs to be confirmed. In any case as pointed out earlier, oncogenic viruses are implicated in the causation of mammary tumours in mice. The C3H strain was produced by selective inbreeding as a high-mammary tumour strain. After the strain had been developed it was discovered that viruses, transmissible in the mothers' milk were the principle cause of the tumours. Foster-nursing of baby female C3H mice by mothers who did not carry the viruses concerned dramatically reduced their risk of developing mammary tumours. This situation has no obvious parallel in man and I find it difficult to believe that inbred mice that carry an oncogenic virus in high titre are a suitable model for assessing cancer risk in man.

In my opinion, therefore the answer to Question 3 is "Yes" and the answers to question 4 is "No".

In the case of diethylstilbestrol there is no evidence that it produces effects which are not also exhibited by natural estrogens such as 17β-estradiol. I can find no logical grounds, therefore, in terms of range of biological activity for regarding diethylstilbestrol as more dangerous because it is an unnatural rather than a natural estrogen. One can reasonably adopt this view in the case of diethylstilbestrol because it has been the subject of many experimental studies. But the same view might be more difficult to sustain in the case of other unnatural agents which have been less studied. For this reason, I personally would generally be a little more suspicious regarding unnatural than natural agents unless adequate biological data were available for scrutiny.

In Table 5, I have outlined an answer to Question 6 which represents my own assessment of the situation. I realise that what I have written begs certain questions, for instance, what does "materially" mean and how does one distinguish "natural activity" and "unnatural activity"? I believe that there are common sense answers to these questions but this is not the place to consider them. The main purpose of Table 5 is to illustrate a tenable philosophical approach to the problem.

Table 5 Is a proposed use of an anabolic agent likely to be safe for man?

Probably YES if:
1. No residue in meat or milk.
2. Residues of natural agents do not materially raise circulating levels of the agents themselves or of endogenous hormones above the maxima found in normal, mature, non-pregnant humans.
3. Residues of unnatural agents do not raise natural hormonal activities above those in normal mature, non-pregnant humans.
Tests required if:
There is any doubt that the activity of an **unnatural** agent is itself **unnatural.**

Before closing I should like to mention two theoretical problems which have so far received little attention. In laboratory animals a most important factor in determining whether and, if so, which tumours will arise in response to a particular hormone is the spectrum of oncogenic virus carried by the test animal. At present we know nothing about the oncogenic viruses carried by man, although we can be pretty sure that he does carry his own spectrum of such agents. If and when viruses which are oncogenic for man are described, we shall have to go back and see whether anabolic agents of the kinds used in meat production influence the risk of development of the kinds of cancer to which these viruses can give rise. The effects we find might, of course, be in either direction.

A related problem is that the administration of anabolic agents to animals is likely to lead to the active proliferation of certain oncogenic viruses in them and these are likely to find their way into meat and possibly milk. We have at present no idea whether this could pose a cancer hazard for those who handle or consume these products. I would not suggest that this is a matter for serious concern at the present time, however, I feel it is an area in which we need more information.

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