

14. Hormones as growth promoters: the precautionary principle or a political risk assessment?

Jim W. Bridges and Olga Bridges

14.1. Introduction

Oestrogenic steroid hormones ('oestrogens') have a crucial role in cellular regulation in all vertebrate species. The levels required to bring about such changes are very low, around 0.1–1 pg/ml (picograms per millilitre) of serum. It has been known for over five decades that oestrogens affect the development of the male reproductive system of mammals (Zuckerman, 1940).

Nonetheless, oestrogenic steroid hormones are often called 'female hormones'. The female reproductive system initially develops independently of the hormone regulatory system. This means that by default an animal is female if hormone stimulation is absent (Wilson and Lasnitzki, 1971). Nonetheless, in both sexes oestrogens are needed for fertility.

At a level above the physiological one, natural oestrogens produced by mammals, such as oestradiol-17 β , have a lasting effect on males. Experimental studies have shown, for example, that the administration of oestradiol-17 β to mice, rats, guinea pigs and rabbits, during both foetal and perinatal life, can result in significant defects in the pituitary–hypothalamic function in males. This, in turn, may disrupt testicular function during adulthood (Takasugi, 1979; Orgebin-Crist *et al.*, 1983; Davies and Danzo, 1981; Brown-Grant *et al.*, 1975).

For maximum growth a combination of both oestrogens and androgens (male hormones) is required. The growth promoting effects are attributed principally to the ability of combinations of oestrogens and androgens to increase the retention of dietary nitrogen through protein synthesis through several mechanisms (European Commission, 1996). After the Second World War, the recognition of the growth promoting properties of oestrogens, either alone or in combination with androgens, led to their introduction as a tool to increase meat production. Diethylstilboestrol (DES), as a cheap, better absorbed analogue of the natural hormone oestradiol-17 β , became the favoured growth

promoter for cattle, sheep and poultry in many countries (Schmidely, 1993).

As with many steroid growth promoters, DES was administered as an implant under the skin of young animals or as a feed additive. In the early 1970s concerns about its safety were raised when DES was confirmed to be a human carcinogen. In the scientific community, however, consensus was reached that the health risk was insignificant. The DES residues in meat were very low (below the limit of analytical detection) compared to those which individuals were exposed to when DES was used as a drug. Use of DES as a growth promoter continued in some Member States of the European Union (EU) longer than in the United States. It was finally banned for this use throughout the EU in 1987 because of uncertainty as to whether there was a definable 'no effect' dose for its potential tumour inducing effects in humans (European Commission, 1996), although some Member States had introduced an earlier ban. In the United States a totally different pattern of events took place. DES was banned initially as a growth promoter in 1972 on the grounds that it was a carcinogen, violating the so-called 1958 Delaney Clause. This clause prohibits food for human consumption that contains carcinogenic substances, but was very difficult to apply in practice because the majority of foods contain trace amounts of carcinogenic substances. However, public opinion ensured that the clause remained on the statute books. The regulators had been forced to refer to 'minimum detectable levels' in justifying their inaction regarding the Delaney Clause in the case of DES. The US Food and Drug Administration (FDA) estimated that the economic burden to the consumers from a DES ban would be approximately USD 500 million per annum. The calculation of this estimate involved a number of questionable assumptions. The estimated health risk was 1 cancer in 133 years (Jakes, 1976).

In 1974 the use of DES as a growth promoter was temporarily reinstated because of

procedural deficiencies in the original bill banning DES in the United States. The farming lobby made strong claims regarding the serious economic consequences of a further ban. These claims were made despite the fact that there were alternative growth promoters already on the US market (see Table 14.1.). The ‘breathing space’ enabled the pharmaceutical industry to develop additional hormonal growth promoters. At the same time a scientific debate was taking place on what residue level of DES could be regarded as without significant risk to human health. In 1976 the FDA set the minimum detectable level of DES (the regulatory level) at 2 ppb (parts per billion). The FDA estimated that levels of DES in meat were of the order of 0.5 ppb (McMartin, 1978), but was not able to verify this estimate by measurements. The safety of oestrogens in the oral contraceptive pill and the high levels of natural oestrogens in pregnant women were cited as crucial evidence of low residues of DES in food being without risk to the consumer. This argument did not take into account that young children with low natural levels of oestrogens were the likely ‘at risk’ group (McMartin, 1978). The FDA also omitted the fact that DES has many structural differences from both oestradiol and the oestrogenic components of the oral contraceptive pill.

In 1979 DES was finally banned because there were no toxicological grounds for identifying a residue level below which a carcinogenic effect would not occur (Jakes, 1976).

The concerns of the high cost to the consumer from a DES ban were probably groundless. When the ban was eventually implemented there was little evidence of a sustained increase in the costs of meat production. In the case of the United States the lack of increase in costs could be explained by the availability of alternative growth promoters and, in part, by the wrong assumptions made in the preliminary calculation of costs. It is worth noting that the FDA has continued to support the use of other oestrogenic compounds as growth promoters in cattle, (including oestradiol, trenbelone and zeranol) because of the perceived importance for the economic efficiency of meat production.

In 1982, an EU expert working group (the Lamming Committee) comprising members of the Scientific Committee for Food and the

Scientific Committee for Animal Nutrition (the lead committee for issues concerning growth promoters), reached an interim conclusion that oestradiol and several other growth promoters with a hormonal action were safe as a growth promoting agent in cattle. This decision was clearly unpopular with the EU officials. Further work was carried out by the Lamming Committee over the next few years, but this did not change its opinion. The committee was disbanded in 1987 and the EU did not publish its interim conclusions. However, members of the committee published their opinions independently of the EU in the scientific literature (Lamming *et al.*, 1987).

In 1988, a risk assessment by the Joint Expert Committee on Food (JECFA) of the World Health Organization (WHO) and the Food and Agriculture Organization reached a similar conclusion to that of the Lamming Committee. JECFA used the standard approach for risk assessments still employed by scientific advisory committees today. JECFA only considered the following (JECFA/WHO, 1988):

- risk when the growth promoters are used according to authorised use (though at the time and since there have been indications of significant accidental or deliberate misuse, which might be expected to lead to higher meat residues);
- individual growth promoters (rather than their combinations);
- data provided by the manufacturers.

Shortly after the publication of JECFA’s conclusions the EU issued a ban not only on the use of oestradiol but also on the use of other natural and synthetic steroid hormones as growth promoters. This ban was first adopted in 1985 but was disputed before the European Court by the United Kingdom. It was annulled because of procedural deficiencies. The ban was finally agreed in 1988. It encompassed a ban on the use of oestradiol 17- β , testosterone, progesterone, zeranol, trenbelone acetate and melengestrol acetate within Member States. In 1989 it was extended to imports from third world countries except where such growth promoters were already banned or countries were operating hormone-free cattle export programmes. This action could be regarded as an application of the precautionary principle, although the principle was not formalised at the time.

It is important to analyse the reasons why the European Commission decided to overrule the views of both committees. Three factors appear to have had a particular influence on the Commission's decision regarding DES's use as a growth promoter:

- firstly, the scientific evidence that DES, which had been extensively used as a growth promoter, caused vaginal clear-cell adenoma in young women (Herbst and Bern, 1988);
- secondly, increasing public concern about the health risks from hormones generally. The first generation oral contraceptive pill was blamed for increased incidence of breast cancer and thrombosis;
- thirdly, several epidemiological studies published at the time claimed that oestrogenic environmental contamination could result in anomalies in growth, sexual development and puberty. In Puerto Rico over 10 000 cases of anomalous sexual development, including premature development of breasts and body hair and pseudo precocious puberty, were reported (Perez-Comas, 1988).

These changes were associated by the authors with high serum total oestrogens. However, the source of the oestrogen contamination was not clearly identified. Similar adverse effects were observed in Italy, believed to result from accidental contamination of food by DES (Fara *et al.*, 1979). In addition, in 1980, analysis of Italian baby food made with homogenised veal showed the presence of DES at significant levels. This was claimed to result from implants that were not removed after slaughtering of animals.

It is evident that the human risk associated with the use of oestradiol and oestradiol-related compounds is governed by many interrelated factors:

- the nature of the growth promoter(s) used, the site and dose administered to cattle and the time period which has elapsed between its administration and the slaughter of the animal;
- the amount of meat and meat products derived from slaughtered animals treated with the growth promoters which is consumed by an individual over an extended period of time;
- indirect contact with substances with oestrogenic properties through environmental contamination or other forms of exposure;

- the susceptibility of the individual consumer.

In addition, there exists a potential for accidental or deliberate misuse of oestradiol and other hormonally related substances in the cattle industry. This misuse may take several forms:

- use of a dose higher than considered acceptable;
- a complex mixture of oestrogenic steroids;
- an inappropriate injection site;
- failure to remove an injection site or an implant (likely to have much higher hormone levels than elsewhere) from a slaughtered animal;
- a shortened withdrawal period;
- use of illegal substances.

Illegal use of growth promoting substances in Member States was the subject of the 1989 Pimenta Report. The report found no evidence in the use of oestradiol-17 β , but nonetheless endorsed the ban because it facilitated controls and consumer confidence in meat. There have been claims that the EU ban on steroid hormones has led to illegal use, not only of the 'safer' steroids but also the more toxic ones, such as DES (Loizzo *et al.*, 1984). In other words, the ban may have led to an increased risk to the consumer, rather than a reduction in risk. In the absence of a substantial regular monitoring programme, the extent of such misuse and the consequent increased risk, if any, to the consumer is hard to gauge. It is noted, however, that DES was detected last year in US meat imported into Switzerland.

In most reported cases of accidental contamination of food by oestrogenic substances, the anomalous effects have been considered to be transient and reversible. However, the long-term effects of exposure of prepubescent children to oestrogenic substances are as yet unknown. The absence of a demonstrable threshold concentration, below which there is no effect, adds to this uncertainty (European Commission, 1996).

14.2. Impacts of oestrogenic compounds on wildlife

This issue was not considered by either the Lamming Committee or JECFA. Two sources of information have emerged on possible impacts of oestrogenic compounds on the environment, namely:

- effects of natural and synthetic oestrogens themselves on endocrine function in wildlife;
- effects of non-steroid chemicals which impact wildlife through endocrine disruption.

No comprehensive studies were conducted until the late 1980s. However, there was sufficient information generated in the 1970s to raise concern about the environmental impacts of compounds with oestrogenic properties. For example, it was reported in 1970 that dichlorodiphenyl trichloroethane (DDT) decreased oestradiol blood levels and deposition of medullar bone (Scientific Committee on Veterinary Measures Relating to Public Health, 1999). It was suggested that the oestrogenic property of DDT was responsible for the observed effect.

Growth reduction was observed in catfish exposed to DES as long ago as 1972, indicating that DES was likely to affect a wide range of species in the environment (Peakall, 1970). Johnstone *et al.* (1978) subsequently described a significant suppression of both length and weight of rainbow trout following dietary administration of oestradiol-17 β (Bulkey, 1972).

A number of chemicals widely present in the environment (DDT, polychlorinated biphenyls (PCBs) and alkylphenols) disrupt oestrogen receptor function in wildlife (Johnstone *et al.*, 1978; Mueller and Kim, 1978; Reijnders, 1986; Bergman and Olsson, 1985; Aulerich *et al.*, 1985). Feminisation of male birds (having both ovarian and testicular tissue) was induced by ethinyloestradiol-17 α (Delong *et al.*, 1973).

The studies cited above were ignored until the late 1980s, when concerns regarding the possible impact on the environment of veterinary drugs and growth promoters were raised. This was discussed in the 1989 Collins Report, but no in-depth evaluation resulted. This may be ascribed to the lack of interest of drug agencies in the environment and to the widely held assumption that any excreted drugs or growth promoters would be in a very diluted form and quickly degrade in the environment. The extent of the environmental impact of the use of oestrogenic growth promoters remains to be established. The recent international workshop on hormones and endocrine disruptors in food and water (in Copenhagen in 2000) confirmed concerns about this issue.

14.3. What were the uncertainties regarding the use of oestrogenic growth promoters for human health?

First, oestrogenic growth promoters were introduced to improve efficiency in cattle production. Human health issues, possible environmental impacts and farm animal welfare were not given significant attention. Histological changes in the prostate and bortholinic gland have been detected in cattle following oestradiol-17 β administration. However, the physiological significance of these changes remains unclear.

Second, residues of the compounds in meat were subsequently measured and shown to be low (in the case of oestradiol-17 β within the 'physiological range'), although what constitutes the physiological range continues to be controversial. The other limitation in these studies was the suitability of the analytical measurements. One aspect of this is the failure to assay metabolites with potential oestrogenic activity, for example oestradiol esters.

The EU has continued to encourage scientific evaluation of the safety of these growth promoters. In 1995 in Brussels the European Commission organised an international conference on growth promoters and meat production, involving the major stakeholder groups. No definitive conclusions were drawn. The expert opinions of the Scientific Committee on Veterinary Measures Relating to Public Health have been published since. The conclusion of both opinions is that further attention needs to be given to the exposure of sensitive populations to these substances because of the possible effects on the immune system, the endocrine system and cancer. The EU is also sponsoring a number of ongoing research projects in this area. JECFA continues to hold to its original view that each of the growth hormones with steroid-like action is safe for the consumer.

Prepubertal boys are identified as being at risk. The reliability of measurements of their endogenous levels and production of oestrogens is very questionable since these levels were close to the limits of detection. It means that any additional exogenous oestrogen represents a relatively high percentage of the total body oestrogen. This is particularly relevant in the context of the

safety criterion applied by the FDA, that intake of any hormone from food should constitute less than 1 % of the individual's daily endogenous production.

Neither possible human exposure via release of oestrogenically active excreta from cattle into the environment, nor possible effects on wildlife, were properly taken into account.

Public concern in Europe was initiated by the association of DES with vaginal clear-cell adenoma and increasing worries about the carcinogenic effects of some hormones. It is pertinent in this regard that recent research has demonstrated that oestradiol-17 β (although a natural hormone), is a genotoxic carcinogen. This finding has further fuelled the debate on what is a safe level.

14.4. Has the approach adopted by the European Commission proved to be sound?

There is a growing consensus that cancer of the breast in women and prostate cancer in men may be promoted by high oestrogen exposure. North America has one of the highest breast cancer rates in the world, while Asia and Africa have much lower rates. The pattern is similar for prostate cancer. It has been noted that women with high levels of oestrogen, particularly free oestrogen, have a higher risk of breast cancer development (Adkins, 1975). Studies on migrant populations indicate this risk is principally due to environmental rather than genetic factors.

There is, so far, no good evidence that the EU ban on the use of growth promoters has protected the health of the public. However, it can also be argued that in those countries where these growth promoters have continued to be used, reliable evidence has not been accumulated on their safety to the consumer. Although potential risks to the environment have been identified, no conclusive evidence that growth promoters constitute a significant environmental risk has been produced.

The case for DES use as a growth promoter was a purely economic one. There was no benefit either for the cattle (or other species to which DES was administered) or for the environment. Agricultural specialists continue to debate the extent of the benefits

of anabolic steroids as growth promoters. The economic consequences of this parting of the ways between the United States and the EU with regard to anabolic steroids as growth promoters are very considerable and have yet to be fully evaluated. The divergence of opinions has led to a trade dispute between the EU and the United States (Henderson *et al.*, 1988). From 1989, the United States took unilaterally retaliatory measures in the form of a 100 % *ad valorem* duty on a variety of EU exports at a value of USD 93 million (ca. EUR 93 million) per annum. In 1996 these measures were withdrawn as not compatible with World Trade Organization (WTO) law. In 1997 the legal basis for the EU ban was challenged by the United States and Canada in front of a WTO panel. The panel found largely against the EU. On appeal, several of the grounds were reversed. The only aspect where the panel's view was upheld was that the EU had in its ban not focused specifically enough on residue in meat of each growth hormone. The dispute raises the issue of where the benefit of doubt should lie. An issue of concern is that attempts to resolve the problem are being made in the absence of any formal mechanisms for trading risks and benefits for the public. Potential environmental impacts or animal welfare issues have not been considered in this dispute.

The EU is currently experiencing sanctions against its exports of the order of GBP 100 million (ca. EUR 160 million) per annum. The success of the United States in the WTO hearings on anabolic steroid growth promoters has encouraged further actions on other products where the EU has adopted a precautionary approach on health grounds. The question is whether, if the EU applies the precautionary principle on health grounds to any product(s), it would prove acceptable to the WTO. Some issues have, however, been clarified by the WTO appeal, namely a sanitary protection measure (including a ban) can be permitted if supported by a risk assessment, even if this:

- is not necessarily quantitative in nature;
- takes into account real world issues such as difficulties in control measures;
- is based on 'qualified and respected sources' (WT/DS, 1997), even if these are in the minority.

Table 14.1. US FDA approval dates for anabolic agents

Source: Henderson et al., 1988

Trade name	Anabolic agent	Approved date
Synovex-S	200 mg progesterone/ 20 mg oestradiol benzoate	20/2/1956
Synovex-H	200 mg testosterone propionate/ 20 mg oestradiol benzoate	16/7/1958
Ralgo	36 mg zeranol Or 12 mg zeranol	5/11/1969
MGA	0.25–0.50 mg/day MGA	3/6/1977

14.5. Overall conclusions

The EU took action in 1985 and again in 1988 to ban growth promoters with a steroid-like action, principally in response to public concern about involuntary and unnatural hormone exposure. This action was not at the time supported by the EU's own scientific committee (the Lamming Committee) nor that of WHO (JECFA). It is appropriate to apply the precautionary principle in those situations where the science base is inadequate to confirm the safety of a product. Neither committee was required to properly characterise the degree of uncertainty in their assessment. As this question was never asked, the original EU ban was, in reality, a political risk assessment.

More recent scientific research, however, probably justifies the application by the EU of the precautionary principle to continue the ban.

The handling of the issue of steroid hormones as growth promoters has wider implications in determining acceptability of the use of chemical substances, namely that:

- it is very important, in arriving at their conclusions, that scientific committees are requested to identify the uncertainties in their assessments;
- rigorous and transparent mechanisms must be developed for evaluating risks against benefits.

Table 14.2. Hormones as growth promoters: early warnings and actions

Source: EEA

1970s	Concerns about growth promoters' safety, as DES confirmed a human carcinogen
1972	Peakal publishes that DES likely to affect a wide range of species in the environment (wildlife) but this was ignored until the late 1980s
1972	DES banned as a hormone growth promoter in the United States
1974	Use of DES reinstated in the United States
1976	US Food and Drug Administration (FDA) sets the minimum detectable level of DES
1979	DES banned again on the grounds of the impossibility of identifying levels below which it would not be carcinogenic
1982	EU expert working group (Lamming Committee) concludes that some growth promoters are safe
1985	First EU ban is adopted, ignoring results from the Lamming Committee because the scope of their assessments had not been broad enough
1987	Lamming Committee disbanded by EU and their results were not published
1988	Ban of several growth promoters throughout the EU based on uncertainty of their effects on humans
1988	WHO/FAO Joint Expert Committee on Food, using standard risk assessments, reaches same conclusions as Lamming Committee
1989	EU ban extended to other growth promoters and to imports from third world countries
1989	Pimenta Report finds illegal use of growth promoters in some Member States
1989-96	USA takes unilateral retaliatory measures on EC exports
1995	European Commission organises an international conference on growth promoters and meat production where uncertainties remain regarding effects on the immune system, endocrine system and cancer
1999	The EU Scientific Committee on Veterinary Measures Relating to Public Health publishes a report concluding that no threshold levels can be defined for six growth promoters
2000	International workshop on hormones and endocrine disrupters in food and water confirms impacts on the environment (wildlife) of veterinary drugs
2001	EU still suffers from sanction to its exports of around EUR 160 million per year

14.6. References

- Adkins, E. K., 1975. 'Hormonal basis of sexual differentiation in the Japanese quail', *Journal of Comparative and Physiological Psychology* Vol. 89, pp. 61–71.
- Aulerich, R. J., Bursian, S. J., Breslin, W. J., Olson, B. A. and Ringer, R. K., 1985. 'Toxicological manifestations of 2,4,5,2,4,5-2,3,6,2,3,6- and 3,4,5,4,5,-hexachlorobiphenyl and Aroclor 1254 in mink', *Journal of Toxicology and Environmental Health* Vol. 15, pp. 63–79.
- Bergman, A. and Olsson, M., 1985. 'Pathology of Baltic ringed seal and grey seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of a widely distributed disease syndrome?', *Finn. Game Research* Vol. 44, pp. 47–62.
- Brown-Grant, K., Fink, G., Greig F. and Murray, M. A., 1975. 'Altered sexual development in male rats after oestrogen administration during the neonatal period', *Journal of Reproduction and Fertility* Vol. 44, pp. 25–42.
- Bulkey, R. V., 1972. 'Diethylstilbestrol in catfish feed', *Trans American Fisheries Society* Vol. 101, pp. 537–599.
- Davies, J. and Danzo, B. J., 1981. 'Hormonally responsive areas of the reproductive system of the male guinea pig II: Effects of estrogens', *Biological Reproduction* Vol. 25, pp. 1149–1158.
- Delong, R. L., Gilmartin, W. G. and Simpson, J. G., 1973. 'Premature births in California sea lions: Association with high organochlorine pollutant residue levels', *Science* Vol. 181, pp. 1168–1170.
- European Commission, 1996. *Scientific Conference on Growth Promoters and Meat Production Proceedings*, Office for Official Publications of the EU Communities, Luxembourg.
- Fara, G. M., Del Corvo, G., Bernuzzi, S., Bigatello, A., Di Pietro, C., Scaglioni, S. and Chiumello, G., 1979. 'Epidemic of breast enlargement in an Italian school', *Lancet* Vol. 11, pp. 295–297.
- Henderson, B. E., Ross, R. and Bernstein, L., 1988. 'Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award lecture', *Cancer Research* Vol. 48, pp. 246–253.
- Herbst, A. L. and Bern, H. A., 1988. *Developmental effects of diethylstilbestrol (DES) in pregnancy*, Thieme-Stratton, New York.
- Jakes, T. H., 1976. 'Diethylstilboestrol in beef production: What is the risk to the consumer?', *Preventive Medicine* Vol. 5, pp. 438–453.
- JECFA/WHO, 1988. *Technical Report Series N7/63*, Geneva.
- Johnstone, R., Simpson, T. H. and Youngson, A. F., 1978. 'Sex reversal in salmonid cultures', *Aquaculture* Vol. 13, pp. 115–134.
- Lamming, G. E., Ballarini, G., Baulieu, E. E., Brooks, P., Elias, P. S., Ferrando, R., Galli, C. L., Heitzman, R. J., Hoffman, B., Karg, H., Meyer, H. H. D., Michel, G., Poulsen, E., Rico, A., van Leuwen F. X. R. and White, D. S., 1987. 'Scientific report on anabolic agents in animal production', *Veterinary Records* Vol. 121, pp. 389–392.
- Loizzo, A., Gatti, G. L., Macri, A., Moretti, G., Ortolani, E. and Palazzesi, S., 1984. 'Italian baby food containing diethylstilboestrol three years later', *Lancet* Vol. 5, pp. 1014–1015.
- McMartin, K. E., 1978. 'Diethylstilboestrol: A review of its toxicity and use of a growth promotant in food producing animals', *Journal of Environmental Pathological Toxicology* Vol. 1, pp. 279–313.
- Mueller, G. G. and Kim, U. H., 1978. 'Displacement of estradiol from oestrogen receptors by simple alkylphenols', *Endocrinology* Vol. 102, pp. 1429–1435.
- Orgebin-Crist, M. C., Eller, B. C. and Danzo, B. J., 1983. 'The effects of estradiol, tamoxifen and testosterone on the weights and histology of the epididymis and accessory sex organs of sexually immature rabbits', *Endocrinology* Vol. 113, pp. 1703–1715.
- Peakall, D. B., 1970. 'p,p'-DDT: Effect on calcium metabolism and concentration of estradiol in blood', *Science* Vol. 168, pp. 592–594.
- Perez-Comas A., 1988. 'Premature sexual development in Puerto Rico', *Boletín*

Asociacion Medica de Puerto Rico Vol. 80, pp. 85–90.

Reijnders, P. J. H., 1986. 'Reproductive failure in common seals feeding on fish from polluted coastal waters', *Nature* Vol. 324, pp. 456–457.

Schmidely, Ph., 1993. 'Revue bibliographique quantitative sur l'utilisation des hormones anabolisantes à action steroïdienne chez les ruminants en production de viande. Performances zootechniques', *Annales de Zootechnie* Vol. 42, pp. 333–360.

Scientific Committee on Veterinary Measures Relating to Public Health, 1999. 'Assessment of potential risks to human health from hormone residues in bovine meat and meat products'.

Takasugi, N., 1979. 'Development of permanently proliferated and cornified vaginal epithelium in mice treated neonatally with steroid hormones and the implication in tumorigenesis', *National Cancer Institute Research Monographs* Vol. 51, pp. 57–66.

Wilson, M. E. and Lasnitzki, I., 1971. 'Dihydrotestosterone found in foetal tissues of the rabbit and rat', *Endocrinology* Vol. 89, pp. 659–668.

World Trade Organization, 1997. WT/DS26/R/USA and WT/DS48/R/CAN.

Zuckerman, S., 1940. 'The histogenesis of tissues sensitive to estrogens', *Biological Review* Vol. 15, pp. 231–271.