This paper has been prepared for discussion by the COT Working Group on Phytoestrogens and does not necessarily reflect the final views of the Group.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

WORKING GROUP ON PHYTOESTROGENS

Diet and the aetiology of temporal advances in Human and Rodent Sexual development. Ashby *et al.* (2000).

Background

1. The FSA funded a study to investigate the estrogenicity of a representative soya-based & cow's milk-based infant formulae on the sexual development in rodents. This work has been recently published as part of a larger project examining the effects of diet on sexual development (Annex 1).

2. Estrogenic activity can be assayed by measuring increases in uterine weight ("uterotrophic activity") in immature or ovariectomised rodents. These models are used as they have low or no production of endogenous estrogens and are therefore, more sensitive to the administration of exogeneous estrogens. Advances in the age at which vaginal opening or first oestrus occurs in rodents is also used as an assay for exogeneous estrogens.

Experimental Design

3. Rats (n=28-29) received either a cows' milk or soya based infant formula via drinking water between post-natal days 21/22 to 24/25 or from post-natal day 21-55. The infant formula was prepared according to manafacturers instructions (termed 100% in paper). The synthetic estrogen, diethylstilbestrol (DES) was administered as a positive control (n=10).

Results

4. Both soya based and cows' milk formula showed uterotrophic activity (wet & dry weight) with soya-based infant formula consistently inducing larger responses (Figure 1 of Annex 1). However consumption of formula was higher in this experimental group. The intake of 4-6 old human infants has been estimated at 4.5-8mg/kg/day. When consumption of soya-infant formulae was normalised to body weight the average intake by rats was approximately 3-fold greater than that estimated for infants. No effects were seen in rodents given a similar calorific quantity of soya infant formulae to that recommended for infants (33% strength). Interestingly, soya-based infant formula did not elicit a uterotrophic response in the adult ovariectomized model.

5. Exposure to soya-based infant formula (100%) between post-natal days 21-55 resulted in advances in vaginal opening and first oestrus which was independent of body weight (Tables 1 of Annex 1). The age of vaginal opening and first oestrus was not determined for cows' milk formula group. A non-significant advance (0.5 days) in vaginal opening was observed in rats' fed 33% soya-based infant formula.

5. A uterotrophic response was induced both by a phytoestrogen containing (soya-based infant formula) and a phytoestrogen free infant formula (cows milk) and phytoestrogen free diet (AIN76A). These results suggest that estrogenic activity could not be conclusively associated with phytoestrogens in the soya based infant formula. Using selective pharmacological inhibition of sex hormone production and action, the mechanisms responsible for the uterotrophic response were examined.

6. The uterotrophic effects of soya based and cows milk formula and AIN76A were abolished when a estrogen receptor blocker (Faslodex FAS) was incorporated into the experiments (see figure 1 of Annex 1). Additionally, a GnRH blocker (ANT)* abolished the uterotrophic activity of the three diets, but not of DES or Estradiol (figure 2 of Annex 1). **ANT blocks GnRH in the hypothalamus to stop production of FSH & LH & concomitant production of estradiol.*

7. Co-administration of an aromatase inhibitor, anastrazole (ANAS) with soya-based infant formula abolished the uterotrophic activity without affecting

food consumption. This suggests that the estrogenic effect is mediated via endogenous production of estradiol rather than a direct estrogenic effect of components of soya milk infant formulae on uterine estrogen receptors.

Conclusions

8. These results indicate that soya based and soya free infant formula can elicit an estrogenic response in a rodent assay. The estrogenic response is not directly associated with the phytoestrogen content of the diet.

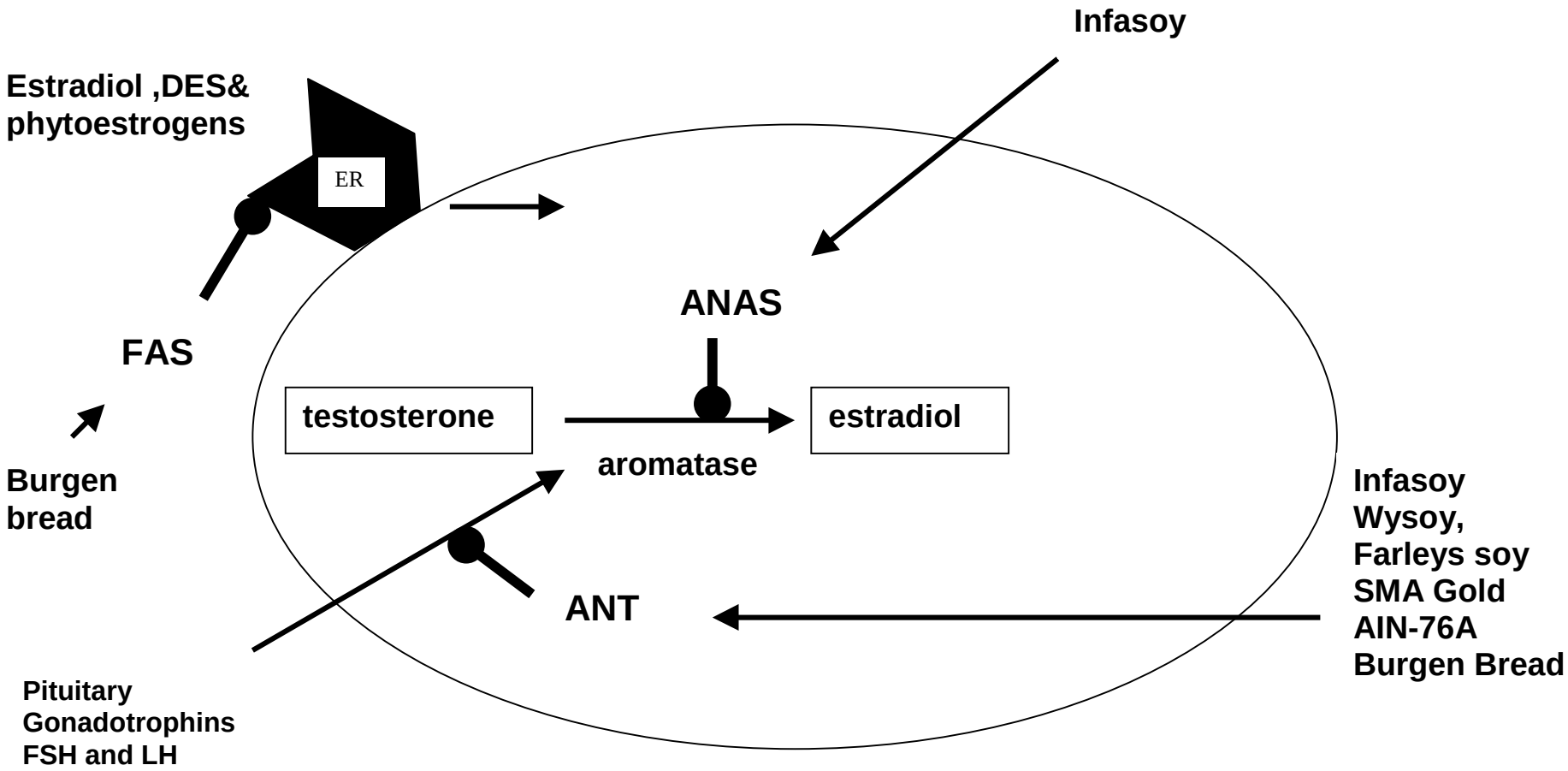

Figure 2: Inhibition of estrogenicity by infant formulae and Burgen bread. Estrogenic activity was determined by measurement of uterine weight, detection of vaginal opening and first oestrus. Inhibition is represented as an oval arrow. ANAS blocked the uterotrophic response to Infasoy indicating that endogenous estrogens mediate this effect. Inhibition by ANT abolished the uterotrophic activities of soy infant formula, SMA gold and AIN-76A implicating the involvement hypothalamus signalling. Uterotrophic activity of Burgen Bread was blocked by FAS, consistent with the phytoestrogen content and their effects on the estrogen receptor. These results indicate that infant formulae & rodent diets produce estrogenic effects at the hypothalamus. DES acts at estrogen receptors in reproductive tissues. Components in Burgen bread act by both mechanisms. The dietary components leading to advanced sexual development have yet to be determined.