Synergy between synthetic oestrogens?

Arnold *et al.*[1] recently reported that a range of chemicals could act synergistically to bind and activate the human oestrogen receptor (hER) *in vitro*. Most markedly, a 1:1 mixture of the insecticides dieldrin and endosulfan produced a 1,000-fold higher activity than when present alone. Arnold *et al.*[1] interpreted this as support for a role of environmental oestrogens in increasing the incidence of human breast and testicular cancers, and decreasing male sperm counts and sperm quality. The chemical selection criteria and the test data have subsequently been discussed and challenged[2–5]. We have evaluated the oestrogenic effects of dieldrin and endosulfan directly using two standard assays. Our data fail to confirm the conclusions of Arnold *et al.*

We used two assays that are sensitive to a wide range of oestrogens. In a yeast hER transactivation assay[6], the hER sequence is incorporated into the yeast genome. Activated oestrogen receptors are detected colorimetrically by a *lacZ* reporter gene. In a uterotrophic assay, sexually immature rats are treated with test chemicals on three successive days, and uterus mass is measured on the following day. Oestrogenic chemicals cause premature growth of the uterus[7]. This latter assay is generally regarded as the most reliable test for oestrogenic activity.

The top dose levels used in the *in vitro* assays were determined by the solubility of the agents in the assay medium and (in the uterotrophic assay) by clinical observations establishing systemic absorption of the test agents and achievement of a maximum tolerated dose. The low dose level of 5 mg kg^{-1} evaluated in the uterotrophic assays was to take account of the possibility that synergism might only be evident with low levels of agonist bound to the receptor. The lowest of the dose levels used in the *in vitro* assays automatically took account of that possibility.

Dieldrin, endosulfan, and a 1:1 mixture of each chemical, were inactive as oestrogens in both assays. These results also established an absence of synergism (Fig. 1). A suppression of gain in body mass caused by the chemicals accounted for the reduced uterus mass seen in some of the uterotrophic assays. These negative results do not support the findings of Arnold *et al.*[1], although it is possible that the lack of oestrogenic activity of dieldrin and endosulfan may have compromised any observation of synergism between them.

Consequently, we evaluated the potential for synergism between dieldrin and methoxychlor, an established oestrogen[7] (Fig. 1). For the dieldrin/methoxychlor mixture experiments a dose level of methoxychlor was selected that gave a submaximal oestrogenic response in each of the assays. No synergism was observed in either of the assays (Fig. 1).

Our results do not support the assertion that synergism between oestrogens is likely to present a major human or wildlife health concern.

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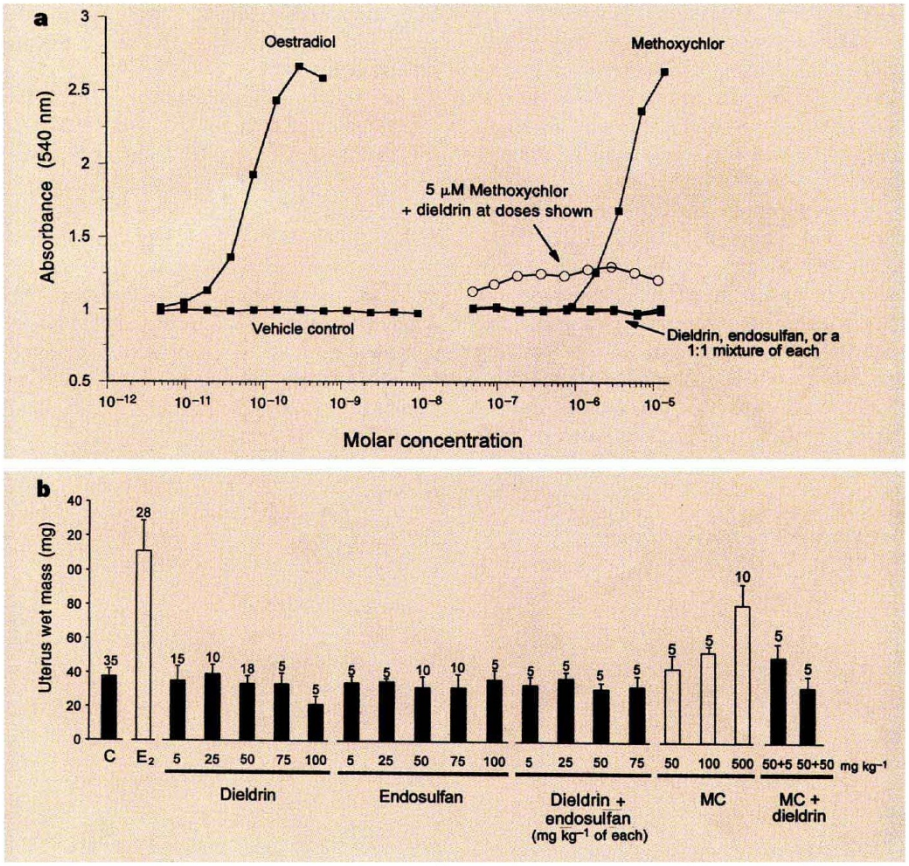

Figure 1 a, **Results of the yeast oestrogenicity assay[6]. Several different commercial samples of the three test chemicals were evaluated, with identical results. Representative data are shown for a sample of dieldrin (Chem Service, West Chester, PA; 98%), a sample of endosulfan (Chem Service; 98%, 60% α-isomer, 38% β-isomer), and a sample of methoxychlor (Sigma; 98%). Test agents were dissolved in ethanol and added to 96 well plates at the concentrations shown. Plates were incubated with the yeast for 4 days at 32 °C (ref. 6). b, Immature Alderley Park (Wistar-derived) rat uterus mass after three daily subcutaneous injections (5 ml kg^{-1}) of arachis oil (control; C), oestradiol (E_2, 50 μg) or the agents shown at the dose levels indicated. Mean results (± s. d.) are shown with animal group sizes above the bars. The results of several experiments are shown. MC, methoxychlor. The positive control data are shown as open bars. Samples were as in a.**

Ant soldiers are not modified queens

On the basis of certain similarities between ant soldiers and the queen (gyne) of the same species, Baroni Urbani and Passera[1] propose that soldiers evolved as a caste independent of workers, and that they originated directly from the queen. Their argument is undermined by a large body of comparative evidence supporting the conventional view that the existence of discrete minor worker and major worker (soldier) morphs simply represents the end point of an evolutionary sequence that begins with the presence of weakly polymorphic workers and passes through an intermediate phase with an expanded, and progressively bimodal, worker-size frequency distribution[2–5]. Morphological divergence between the worker castes is further accentuated by allometric growth[3].

For each of the three ant genera cited by Baroni Urbani and Passera (*Camponotus*, *Pheidole* and *Zacryptocerus*), in which some