COMMUNICATIONS

Uncoupling of Oxidative Phosphorylation in Intact Mites and in Isolated Mite Mitochondria by a New Acaricide, 5,6-Dichloro-1-phenyloxy-carbonyl-2-trifluoromethylbenzimidazole

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The new acaricide is hydrolysed by water to give 5,6-dichloro-2-trifluoromethylbenzimidazole, which is an uncoupler of mammalian and insect mitochondria (Büchel, Korte & Beechey, 1965; Beechey, 1966; Jones & Watson, 1967; Ilivicky & Casida, 1969). The acaricide would therefore be expected to act as an uncoupling agent. The following evidence suggests that this is the mode of action of the compound:

(a) The acaricide and its hydrolysis product uncoupled rat liver mitochondria with the same activity (the minimum uncoupling concentration was approx. $2\mu\text{M}$ with 2mg of mitochondrial protein present in an oxygen electrode assay), but the acaricide itself only exerted its full stimulatory effect on oxygen uptake after a lag period.

(b) Mitochondria were isolated with difficulty from the greenhouse red spider mite (*Tetranychus telarius* L.) essentially by the method developed for insects by Ilivicky, Chefurka & Casida (1967). This is believed to be the first reported isolation of mite mitochondria. Using pyruvate as substrate, we have established that the isolated mite mitochondria show a respiratory control value of approx. 2, and that they are uncoupled by both the acaricide and its hydrolysis product. As with the rat liver mitochondria, the minimum uncoupling concentration was about $2\mu\text{M}$, and the acaricide only exerted its full uncoupling effect after a lag period.

(c) If an acaricide kills mites through an uncoupling effect on oxidative phosphorylation, the mites should show an increase in respiratory rate before dying. This was tested by immobilizing mites by sticking them to the outside of a roll of sticky tape, which was then placed over the centre well of a Warburg flask. After the initial rate of oxygen uptake had been measured the rolls were removed and the mites treated with chemical. Both the acaricide and its hydrolysis product stimulated respiration by approx. 70%. Suitable controls did not show this stimulation.

In agricultural practice the acaricide is greatly superior to its hydrolysis product in mite control and in its safety to crops. Presumably this is because the lipophilic nature of the acaricide causes it to be concentrated in the plant cuticle, which is

eaten by mites. The ensuing hydrolysis of the acaricide inside the mites will cause death by uncoupling.

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The Mode of Action and Metabolism of 6-Chloro-2-trifluoromethylimidazo[4,5-b]pyridine, an Experimental Herbicide

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The title compound, and a close relative, 2-*tert*-butyl-6-chloroimidazo[4,5-*b*]pyridine, are powerful photosynthetic inhibitors *in vitro*. The Hill reaction was measured with maize and pea chloroplasts by using (i) the oxygen electrode and (ii) ferri-cyanide reduction measured spectrophotometrically. The compounds caused 50% inhibition of the Hill reaction at 1.5 and $2.4\mu\text{M}$ respectively. Preliminary experiments suggest that there is no uncoupling of either cyclic or non-cyclic photophosphorylation at these concentrations. The obvious corollary that the compounds are herbicidal by inhibiting photosynthetic electron flow was tested by keeping the treated plants in the light, or in the dark, by using a simple home-made growth cabinet. The herbicidal effect on mustard, maize and tomato was the same in the light as in the dark for both compounds. We conclude that the herbicidal action of the compounds was not due to interference with photosynthesis.

Tests with mitochondria (rat liver, pea and cauliflower) by using (i) Warburg manometry and (ii) the oxygen electrode showed that the title compound was a moderately active uncoupler of oxidative phosphorylation (minimum uncoupling concentration was about $50\mu\text{M}$), but that the *tert*-butyl compound was not an uncoupler at $100\mu\text{M}$. Since it is probable that both compounds act in the same way, uncoupling of oxidative phosphorylation must also be ruled out as a herbicidal mechanism.

Preliminary metabolic studies have been carried out on the title compound labelled with ^{14}C in the